EXHIBIT A

LL&E notebook 1 PJ

TITLE

PROJECT NO.

BOOK NO.

11

DUPLICA

Laser diode now in use:
NVG Inc. 640nm
5 mW laser
ordered from Digi-K 70-90 milliamps
NVG part no. M6405I

Also, think about using super-bright LED !!

Now trying pyrex tube flow cell (instead of the soft glass previously used) to better match index of refraction w epoxy.

SIGNATURE

5/3/99

DISCLOSED TO AND UNDERSTOOD

Richard W Shoenfeld

DATE 7/5/99

WITNESS

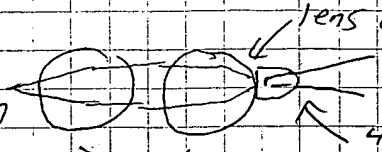
DATE

TITLE

PROJECT NO.
BOOK NO.

12.

Flow Fluorescein system 480nm LED



Illumination with blue LED

Fluoresces at ~~58~~ 520nm

use Edscorp 520nm interference filter for imaging

ordered 8mm BK-7 spheres from Edscorp

SCIENTIFIC SUNDRY PRODUCTS CHICAGO

SIGNATURE [signature] DATE 5/10/99

DISCLOSED TO AND UNDERSTOOD BY Richard W. Monteith DATE 7/5/99 WITNESS DATE

TITLE

PROJECT NO.

BOOK NO.

12

DUPLICAT.

OMEGA ® OPTICAL

FLUORESCENCE SET: XF25

FOR DYES: RH 414, RD1, Fura Red™/ Fluo-3, FM1-43, CR6G, Calcein BODIPY®-Ceramide, Nile Red, fluo-3, FITC, BODIPY®-FL, BCECF, DiO, YOYO™-1, Calcium Green™, Y/G FluoSpheres®, TOTO™-1, rhodamine 123, ethidium-homodimer

EXCITATION: 485DF22 (FULL LINE)

DICHROIC: 505DRLPO2 AT 45° AOI (CHAIN LINE)

EMISSION: OG530 (BROKEN LINE) — we use this

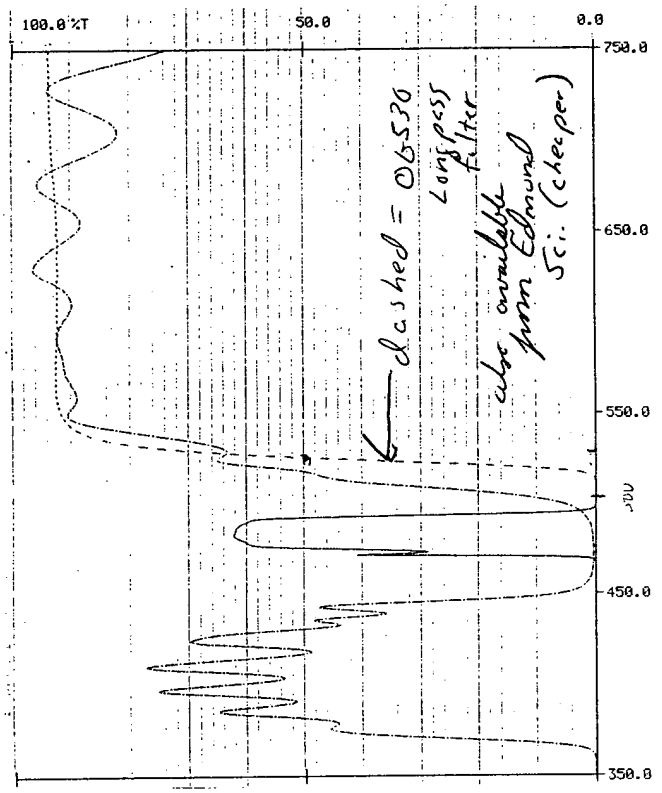
SPECTRAL CONTROL:

EXCITATION: 0.8 CWL TO FAR IR X-AXIS: 350-750 nm X-SCALE: 10 nm/div.

EMISSION: X-RAY TO 1.2 x CWL Y-AXIS: 0-100%T

Arrows on excitation and emission filters point in the direction of light path

Not recommended for use with the following light source(s): Hg



CLEANING OF OPTICAL COMPONENTS
Hold by edges only. First, remove any foreign particles with a puff of dry air. Wipe gently with a soft, lint-free cloth. A final wipe with a few drops of pure anhydrous alcohol will result in a clean, undamaged component.

OMEGA OPTICAL, INC. • TEL: (802) 254-2690 • FAX: (802) 254 3937

Long-pass fluorescence filter

SIGNATURE

DATE 6/9/99

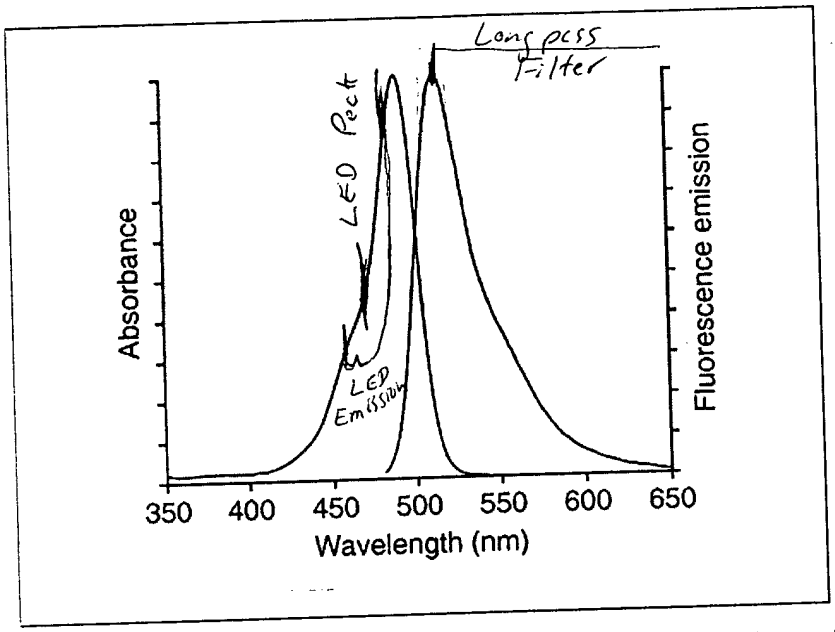
DISCLOSED TO AND UNDERSTOOD BY Richard N Haverfield DATE 7/5/99

WITNESS

DATE

Spectra

Absorption and fluorescence emission spectra of fluorescein (F-1300) in pH 9.0 buffer.



From Molecular Probes Web Site

LED emits at 470 nm! (minimum)
with $\Delta\lambda$ = 30 nm

SCIENTIFIC BINDERY PRODUCTS CHICAGO

SIGNATURE DATE 6/9/99

DISCLOSED TO AND UNDERSTOOD BY Richard V Thurston DATE 7/5/99 WITNESS DATE

TITLE

PROJECT NO.

BOOK NO. 7/8/88

1

To run biomage in IDL

cd, 'c:\dan' get into "home" directory

compile biomage

@test

We measured Argon laser power:

0.5 mw minimum pot. setting

& 9 mw max

Using power meter calibrated at 628 nm.

These estimates are not corrected.

with new coherent power meter from Edmund

we get 20 mw max.

2 mw min.

Tested blue greed Nichia LED with

490-495 nm peak (10 ma)

1 mw power at 100 ma current

at 200 ma device is destroyed

We might run at 100 ma.

SIGNATURE

DATE 7/8/88

DISCLOSED TO AND UNDERSTOOD BY

Michael Hunt

DATE 9/12/02

WITNESS

DATE

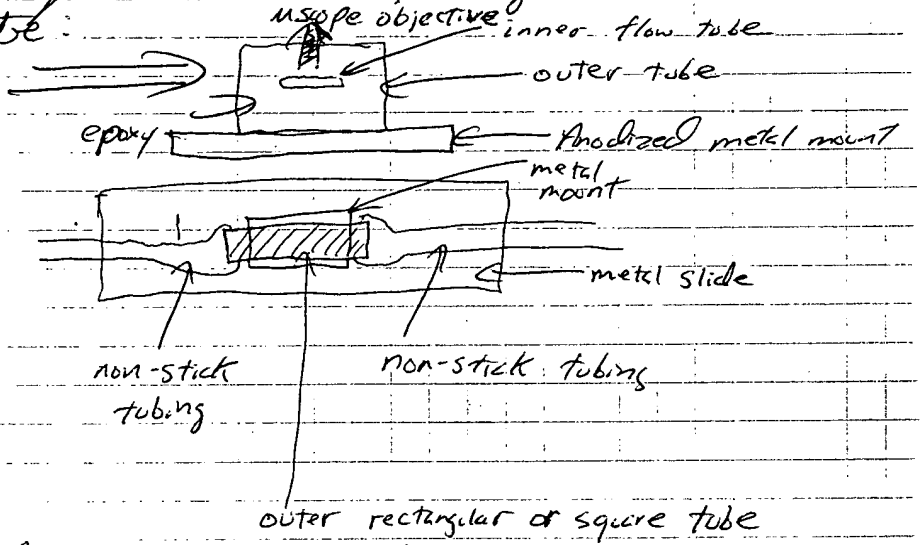
TITLE To Do / Improvements PROJECT NO. BOOK NO. 231

DUPLICATE

Note: 1. to prevent bacteria clumping after centrifuge step — think about using a "anti-clumping" buffer.

2. Build mechanical stage for microscope w easy mounting and adjustment.

3 Build flow cell with rectangular outer tube:



index match glass and epoxy

4. Build LED collimation / focusing module.

5. Perform statistical tests on bead photometry w BIOCOUNT (improved).

6. Calibrate CCD gain + dark + readout noise
Use slope of variance/exposure curve to calibrate gain

7. Find dark noise in TDI mode along columns and different rate from imaging dark noise.

SIGNATURE — 10/1/S

DISCLOSED TO AND UNDERSTOOD BY: Michael Lund DATE 9/11/00 WITNESS DATE

EXHIBIT B

September 30, 1999

J. Winslow Young
Registered Patent Agent
P.O. Box 1088
Centerville, UT 84014-5088

Dear Winslow,

Here is an overview of my invention concerning the use of LEDs for flow cytometry in general and CCD/TDI flow cytometry in particular. Please retain a copy of this for your files.

Use of LEDs in CCD/TDI flow cytometer.

The use of LEDs is practical in flow cytometry, especially for some applications, and has the following advantages:

1. LEDs are typically monochromatic and some can currently output radiant power in the 1 mW to 2 mW range. This power range makes them suitable for some immunofluorescent flow cytometry.

2. LEDs are typically very inexpensive and can be purchased for ~1 dollar, making them extremely attractive relative to lasers or incandescent sources.

3. Their low cost and small size makes it easy to replace an LED of a given wavelength with one of another wavelength, in modular fashion. Since a variety of manufacturers make LEDs at wavelengths that nearly cover the entire visible spectrum, it is nearly possible to match any given visible wavelength fluorescent tag to a superbright LED, allowing the fabrication of a general purpose flow cytometer with a large number of inexpensive LED modules.

4. Simple optics (lenses) can be used to focus the LED beam to a smaller beam size permitting a high-intensity illumination spot.

LEDs haven't been previously used for flow cytometry for the following reasons:

1. Many flow cytometers have higher powered lasers (20 to 100 mW) as illumination sources as they are more forgiving of inefficient optical design.

2. It is common for applications requiring precision photometric measurements of particles in a flow cytometer to use the inner part of the Gaussian beam (say, ~5 to 10% of the power) which has a relatively constant or "flat" intensity to illuminate the core flow. This insures that a particle, no matter where it is in the core flow, will receive almost precisely the same illumination. In systems where constant illumination is not critical (e.g. where one simply wants to detect bacteria and not perform precision photometry) LED illumination becomes practical.

We (and others) have shown that flow cytometry can be performed with a beam power of ~2 mW. We have measured the output power of several superbright LEDs and found them to be ~1 mW of radiant power. The trend towards brighter and more efficient LEDs will only improve the performance and cost of LEDs in the future.

Sincerely,

Paul E. Johnson
President, SoftRay Inc.
519 South 5th St.
Laramie WY 82070

P.S. I would also add that the increasing number of wavelengths, the increasing power, and the recent advent of blue LEDs, makes them particularly suitable just now.

EXHIBIT C

SoftRay, Inc. Confidential

Oct 12, 1999
~~September 30, 1999~~

J. Winslow Young
Registered Patent Agent
P.O. Box 1088
Centerville, UT 84014-5088

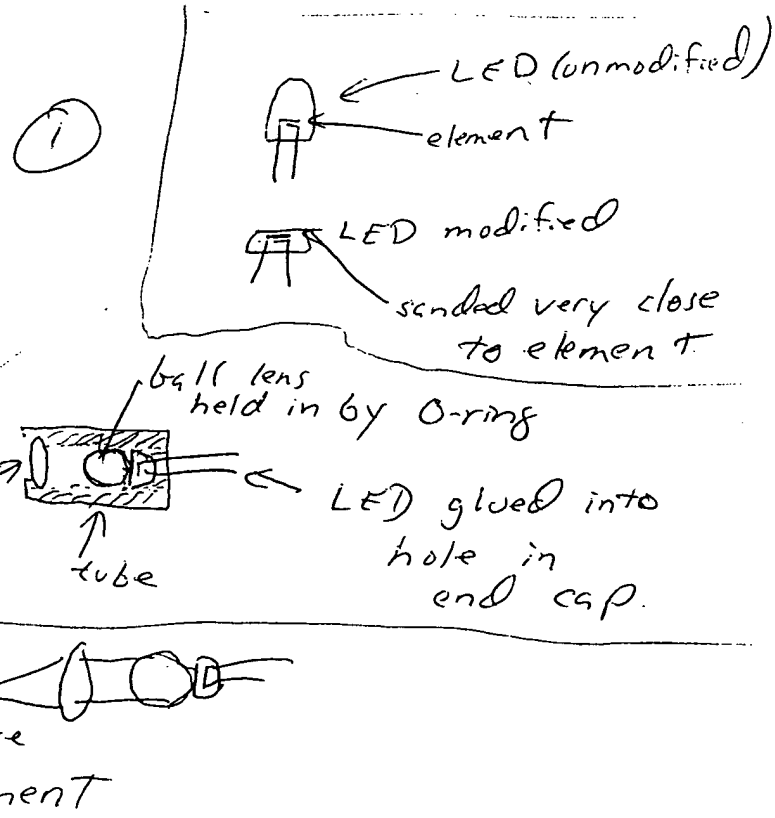
Dear Winslow,

Here is a drawing concerning the use of an LED illumination source in flow cytometry in general and CCD/TDI flow cytometry in particular. Please retain a copy of this for your files.

Also note: I sent a "non-disclosure packet" to B&D, which arrived two days before my visit. This included the letter that I previously faxed to you regarding LEDs and flow cytometry. When I arrived, the Director of New Technology, Bob Hoffman, informed me that they hadn't read the document on LEDs (referred to in a cover letter), and didn't want to, because it interferes with their intellectual property. Interesting. They had me remove this letter from the opened express mail packet in their possession. Neither party signed or had witnessed any of this.

<u>Use of LEDs in Flow Cytometry</u>

In the device below, there are three components, an off-the-shelf LED, a collimating lens, and a focusing lens. I have drawn a ball lens as the collimating lens because it has a fast f/# and can efficiently collect light from the LED. It is commonly used to collect and collimate light from fiber optics. It is most efficient if it makes contact with the LED. The domed surface of an LED acts as a lens, making the LED more directional. I remove this by sawing it off and polishing the end flat with sandpaper or emery paper. The final component is a focusing lens, the size and focal length can be chosen to control the size of the module and the magnification of the final spot. (The magnification is simply the ratio of the focal lengths of the two lenses: f.l.(focusing lens)/f.l.(ball lens).



My best,

Paul Johnson
SoftRay Inc.

EXHIBIT D

Purchase Order 040400A

SoftRay Inc.
519 South 5th Street
Laramie, WY 82070
Telephone: (307)- 745-3743
Fax: (307)-745-3743

To: Nichia
Date: April 4, 2000
Attention: Michael Stewart

ITEM	Description	Number of Units	Unit Price	Price
I	NSPG300A – F Rank (510-520 nm)	10	$6 Ea.	$60
Shipping (est.)	**Fedex C.O.D.**			**$14.50**
TOTAL				**$64.50**

Paul E. Johnson
President, SoftRay, Inc.

EXHIBIT E

Status: U
Date: Fri, 01 Nov 2002 15:11:57 -0700
From: "Paul E. Johnson" <PJohnson@uwyo.edu>
Subject: FW: Respons to NSF 4/28/00
To: bales@mbj-law.com
Thread-Topic: Respons to NSF 4/28/00
Thread-Index: Ab+xaH4luQ1p0h1REdSErQCqAN2PpYWhE9kQAABROwA=
X-OriginalArrivalTime: 01 Nov 2002 22:12:00.0199 (UTC)
FILETIME=[B31F1570:01C281F3]

-----Original Message-----
From: Paul E. Johnson
Sent: Friday, November 01, 2002 3:03 PM
To: Paul E. Johnson
Subject: FW: Respons to NSF 4/28/00

-----Original Message-----
From: Richard W. Shorthill [mailto:shorth@eng.utah.edu]
Sent: Friday, April 28, 2000 5:20 PM
To: Paul E. Johnson
Subject: Respons to NSF 4/28/00

Paul:

4/28/00, 5:20PM
Some items need to be submitted to NSF "Division of Contracts, Policy
and
Oversight
Cost Analysis and Audit Resolution Branch" that we discussed also the
last item
Indirect Costs: . (See sample indirect cost proposal found at Appendix 6

of the Guide.). Call me with questions.

Dick

Richard W. Shorthill, Ph. D.
University of Utah
Mechanical Engineering
50 S. Campus Drive
Salt Lake City, UT 84124-9208
Office E-mail: shorth@eng.utah.edu
Home E-mail: srw4@uswest.net
FAX: 801-585-9826
Phone: 801-581-8623

Content-type: application/applefile; name=bruce#5.doc
Content-disposition: attachment; filename=bruce#5.doc
Content-description: bruce#5.doc

bruce#5.doc

HAMILTON: "Administrative and Financial Information for Potential SBIR/STTR Phase II Awards" answers to the questions for "5" below

5. Provide documentation as detailed below to support the proposed costs in the following categories. (In the event, you have already provided any of the requested documentation; there is no need to resubmit it.)

Senior Personnel: (1) Provide current payroll register for each employee.
(1) Payroll register for SoftRay, Incorporated: Dr. E. Johnson, Ph. D., President [Rate: $34.61/hr]. Dr. Richard W. Shorthill, Ph. D., Senior Scientist and Principal Investigator [Rate: $23.08/hr].

(2) For each named individual who is not presently employed by the organization, provide an employment agreement stating the rate of payment and also a statement setting forth the organization's considerations in determining the rate of pay offered. (Provide salary survey or other documentation to support proposed rate.)

(3) For positions where specific individuals have not been named, provide an explanation of how the rates were determined and any related documentation.
(3) Employees to be hired by SoftRay, Incorporated after award received: Biologist [Rate $13.46/hr] based on undergraduate typical rates for a Biologist/ microbiologist in Laramie, Wyoming.
Graduate student [Rate $15.00/hr] part time employee, SoftRay, Inc. typical rate for starting part time help.

Other Personnel: For all "other personnel" proposed at an annualized salary in excess of $40,000, provide cost data as described for senior personnel above.

Fringe Benefits: Provide the categories of costs (FICA, health benefits, etc.) included and the related amounts. Provide calculation of the actual fringe rate for previously completed fiscal year (total fringe costs / total labor costs).
Fringe Benefits: FICA 7.65%; Unemployment 0.5%; Health insurance, dental care, life insurance, eye glasses, other reasonable and necessary medical procedures; receipts required; The total for all these benefits may not exceed 28% of wages.

Permanent Equipment: (Defined as nonexpendable, tangible personal property, having a useful life of more than one year and an acquisition cost of $5,000 or more per unit.) Provide cost data from three sources which can be in the form of written quotations and/or copies of pricing information contained in catalogues, trade journals, etc. In situations where equipment can only be provided by one specific source provide a sole source justification and a written quote from this source.
No single item $5000 or more dollars.

Travel: Provide an itemization of travel which should include the destination, purpose of travel, number of days in travel status, and the estimated costs for items included in the amount (airfare, cab fare, car rental, per diem rates, hotel and other incidentals.)

Travel

E. TRAVEL		1. DOMESTIC				10425
		2. FOREIGN				
name	Purpose	Location	#trips	$ ea	total	
1 RWS	Project meetings, Soft Ray,Laramie 3 days	SLC/LAR	11	475	5225	
2 PEJ	Technical meeting on Cytometry 4 days	LAR/BOS	1	1400	1400	
3 RWS	Consultant's lab, Bozeman, MT 3 days	LAR/Bozeman	1	900	900	
4 PEJ	Project meetings, Salt Lake City,UT 3 days	LAR/SLC	2	475	950	
5 PEJ	Support meeting w/Becton Dickenson 3 days	LAR/SFO	1	1000	1000	
6 PEJ	Consultant's lab, Bozeman, MT 3 days	LAR/Bozeman	1	950	950	

E. TRAVEL		1. DOMESTIC				$ 11,150
		2. FOREIGN				
name	Purpose	Location	#trips	$ ea	total	
1 RWS	Project meetings, Soft Ray, Laramie 3 days	SLC/LAR	10	475	4750	
2 PEJ	Technical meeting on Cytometry 5 days	LAR/SFO	1	1400	1400	
3 RWS	Consultant's lab, Bozeman, MT 3 days	SLC/Bozeman	1	900	900	
4 PEJ	Present Paper on Cytometry 4days	LAR/BOS	1	1200	1200	
5 PEJ	Project meetings, Salt Lake City,UT 3 days	LAR/SLC	2	475	950	
6 PEJ	Support meeting w/Becton Dickinson 3 days	LAR/SFO	1	1000	1000	
7 PEJ	Consultant's lab, Bozeman, MT 3 days	LAR/Bozeman	1	950	950	

Airfare- coach: LAR/Bozeman, LAR/SFO, LAR/BOS, Private Vehicle-SLC/LAR, LAR/SLC, SLC/BOZ, BOZ/SLC, LAR/SLC. Lodging-actual, per diem- $35/day. Mileage- $0.31/mile. Cab/Bus Fare-actual. Receipt required for items $10 or over.

Materials and Supplies: Provide an itemized listing of materials and supplies. Provide quotations or other appropriate documentation to support any single material and supply item with an extended amount in excess of $5,000.

Materials:

1. MATERIALS AND SUPPLIES 1st YEAR		22200
1.1 Microscope fluorescence conversion modules	5000	

1.2 Improved TDI/CCD camera	2500	[redacted]
1.3 Optics:collimators, focusing, reimaging, mirrors	2800	
1.4 Computer	2000	
1.5 Pump: Liquid	400	
1.6 Laser: LED, gas, dye	2000	
1.7 Chemical/biological: labeled antibodies, labeled beads	3500	
1.8 Filters: interference, $1000 notch m$1000	2000	
1.9 IDL: Programming Interactive data language	1500	
1.10 Bacteria: simulants	500	

1. MATERIALS AND SUPPLIES 2nd Year		$ 4,840
1.1 Chemical/biological: markers, dyes, beads	1200	[redacted]
1.2 Optics: filters(interference, notch), mirrors,	1000	
1.3 Bacteria: simulants	640	
1.4 Laser	2000	

Publication Costs/Documentation/Dissemination: Provide an explanation of items included in this amount and the per item cost (e.g., the estimated number of pages and the per page cost.)

No cost

Consultant Services: For each consultant, provide an agreement which includes the following information: (1) the services to be provided; (2) the period of performance and the consultant's availability; (3) the qualifications of the consultant to perform the work; and (4) the rate of pay (not to exceed $453 per day). (Consultants should be chosen using a competitive selection process; otherwise, sole source justifications should be maintained.)

3.CONSULTANT SERVICES 1st YEAR				$7,950
3.1 Douglas A. Christensen, Ph. D. (EE/Bio. Elec. Engr) specific work in Bioengineering, Laser technology	16hrs	$75/hr	1200	[redacted]
3.2 James J. Smith, Ph. D. (Microbiologist)	66hrs	$75/hr	4950	

	Experimental Design and review				
3.3	Warrie Means, Ph. D. (Meat Scientist)	24hrs	$75/hr	1800	
	", testing meat samples				

3.CONSULTANT SERVICES 2nd YEAR				$7,950
3.1	Douglas A. Christensen, Ph. D. (EE/Bio. Elec. Engr)	12hrs	$75/hr	900
3.2	James J. Smith, Ph. D. (Microbiologist)	66hrs	$75/hr	4950
3.3	Warrie Means, Ph. D. (Meat Scientist)	28hrs	$75/hr	2100

Each consultant will work not more than 6 hours in any day ($450) which does not exceed $453. The original proposal includes their letter of commitment and a resume which demonstrates their qualifications as a consult on this proposal.

Subawards: For each subaward agreement provide (1) the services to be provided, the names of individuals expected to perform the work, and the expected level of effort for each individual; (2) cost information broken out by cost category (salaries, fringe, travel,

Subaward to the University of Wyoming

Personnel:

A. SENIOR PERSONAL: PI/PD 1st YEAR			Period of performance	Funds
1	**Dr. Paul E. Johnson** Direct the subcontract activities	**PD**	3 months of activity over 1 year	0
2	**(To be hired)** Provide mechanical and electronic design for the Cytometer, controls, and tests of the cytometer system	**Engineer**	9 months of activity over 1 year	$21,016

B. OTHER PERSONNEL 1st YEAR		
3. GRADUATE STUDENT Assist the Engineer particularly with the testing of system	600 hours	$9,000
4. UNDERGRADUATE STUDENT Carry out work assigned and work with the engineer and graduate student	500 hours	$5,000

G. OTHER DIRECT COSTS 1st YEAR			
1. MATERIALS AND SUPPLIES			$7,000
1.1 Mech .& Optical Shop (design con - struction of cytometer, optics and controls)	112 hrs	$5,600	
1.2 Graduate student tuition (requirement of the University of Wyoming)		$1,400	

A. SENIOR PERSONAL: PI/PD 2nd YEAR			Period of performance	Funds
1	**Paul E. Johnson**	**PD**	3 months of activity over 1	0

			year	
2	**(To be hired)** Provide mechanical and electronic design for the Cytometer, controls, and tests of the cytometer system	**Engineer**	12 months	$28,020

B	4. UNDERGRADUATE STUDENT 2nd YEAR Carry out work assigned and work with the engineer and graduate student	500 hours	$5,000

G.	OTHER DIRECT COSTS		
	1.1 Mech .& Optical Shop (design con - struction of cytometer, optics and controls)	112 hrs	$5,600

For Year 1 and Year 2, <u>Fringe Benefits:</u> FICA 7.65%; Unemployment 0.5%; Health insurance, dental care, life insurance, eye glasses, other reasonable and necessary medical procedures; receipts required; The total for all these benefits may not exceed 28% of wages.

The total budgets for the subaward are shown in the first and second year budgets in the original proposal.

Indirect Costs: Provide a current indirect cost rate agreement negotiated by a Federal Agency. In the absence of a current negotiated rate agreement, provide an indirect cost proposal and the related financial statements (income statement and balance sheet) for the two most recently completed annual accounting fiscal periods. If an organization has not had financial activity for these periods, indirect cost proposals should be based on what activities the organization expects during the proposed award period. (See sample indirect cost proposal found at Appendix 6 of the Guide.)

In order to avoid delays in the review and processing of your proposal, please send the requested information within 15 days of the above date to:

To: National Science Foundation
Division of Contracts, Policy and Oversight
Cost Analysis and Audit Resolution Branch
4201 Wilson Boulevard, Room 475
Arlington, VA 22230

Cc: to Bruce Hamilton

Questions related to Items 1 and 2 should be directed to the Division of Grants and Agreements (Attn: Andrea Kline) at 703-306-1212. Questions related to Items 3 through 5 should be directed to the Cost Analysis and Audit Resolution Branch (Attn: Patricia Farrell) at 703-306-1244.

EXHIBIT F

Status: U
Date: Fri, 01 Nov 2002 15:11:49 -0700
From: "Paul E. Johnson" <PJohnson@uwyo.edu>
Subject: email correspondence on LEDs
To: bales@mbj-law.com
Thread-Topic: Bruce #5 Friday 5/5/00
Thread-Index: Ab+2q0sEhBG7sSKYEdSErQCqAN2PpYWWjaFgAABeeuA=
X-OriginalArrivalTime: 01 Nov 2002 22:11:49.0746 (UTC)
FILETIME=[ACE41520:01C281F3]

Jennifer,

This is the first of 4 emails that I sent re: LEDs.

Paul

-----Original Message-----
From: Paul E. Johnson
Sent: Friday, November 01, 2002 3:01 PM
To: Paul E. Johnson
Subject: FW: Bruce #5 Friday 5/5/00

-----Original Message-----
From: Richard W. Shorthill [mailto:shorth@eng.utah.edu]
Sent: Friday, May 05, 2000 9:58 AM
To: Paul E. Johnson
Subject: Bruce #5 Friday 5/5/00

Paul:

The #5 items response to Bruce Hamiltons request. Call me at home over the weekend if you have questions.

Dick

Richard W. Shorthill, Ph. D.
University of Utah
Mechanical Engineering
50 S. Campus Drive
Salt Lake City, UT 84124-9208
Office E-mail: shorth@eng.utah.edu
Home E-mail: srw4@uswest.net
FAX: 801-585-9826
Phone: 801-581-8623

Content-type: application/applefile; name=CorNSF5.doc
Content-disposition: attachment; filename=CorNSF5.doc
Content-description: CorNSF5.doc

CorNSF5.doc

Response to BRUCE HAMILTON: "Administrative and Financial Information for Potential SBIR/STTR Phase II Awards" answers to the questions for "5" below

5. **Provide documentation** as detailed below to support the proposed costs in the following categories. (In the event, you have already provided any of the requested documentation; there is no need to resubmit it.)

Senior Personnel: (1) Provide current payroll register for each employee.
(1) Payroll register for SoftRay, Incorporated: Dr. E. Johnson, Ph. D., President [Rate: $34.61/hr]. Dr. Richard W. Shorthill, Ph. D., Senior Scientist and Principal Investigator [Rate: $23.08/hr].

(2) For each named individual who is not presently employed by the organization, provide an employment agreement stating the rate of payment and also a statement setting forth the organization's considerations in determining the rate of pay offered. (Provide salary survey or other documentation to support proposed rate.)

(3) For positions where specific individuals have not been named, provide an explanation of how the rates were determined and any related documentation.

(3) Employees to be hired by SoftRay, Incorporated after award received: <u>Biologist</u> [Rate $13.46/hr] based on undergraduate typical rates for a Biologist/ microbiologist in Laramie, Wyoming.
<u>Graduate student</u> [Rate $12.00/hr] part time employee, SoftRay, Inc. typical rate for starting part time help.

Other Personnel: For all "other personnel" proposed at an annualized salary in excess of $40,000, provide cost data as described for senior personnel above.

Fringe Benefits: Provide the categories of costs (FICA, health benefits, etc.) included and the related amounts. Provide calculation of the actual fringe rate for previously completed fiscal year (total fringe costs / total labor costs).

<u>Fringe Benefits:</u> FICA 7.65%; Unemployment 1.5%; WORKMANS COMP., Health insurance, dental care, life insurance, eye glasses, other reasonable and necessary medical procedures; receipts required; The total for all these benefits may not exceed 28% of wages.

Permanent Equipment: (Defined as nonexpendable, tangible personal property, having a useful life of more than one year and an acquisition cost of $5,000 or more per unit.) Provide cost data from three sources which can be in the form of written quotations and/or copies of pricing information contained in catalogues, trade journals, etc. In situations where equipment can only be provided by one specific source provide a sole source justification and a written quote from this source.

No single item $5000 or more dollars.

Travel: Provide an itemization of travel which should include the destination, purpose of travel, number of days in travel status, and the estimated costs for items included in the amount (airfare, cab fare, car rental, per diem rates, hotel and other incidentals.)

Travel

E. TRAVEL		1. DOMESTIC				10425
		2. FOREIGN				
name	Purpose	Location	#trips	$ ea	total	
1 RWS	Project meetings, Soft Ray,Laramie 3 days. Discuss progress & plans.	SLC/LAR	11	475	5225	
2 PEJ	Technical meeting on Cytometry 4 days. Attend technical papers.	LAR/BOS	1	1400	1400	
3 RWS	Consultant's lab, Bozeman, MT 3 days. Review microbiology tests.	LAR/Bozeman	1	900	900	
4 PEJ	Project meetings, Salt Lake City,UT 3 days. Discuss progress & plans.	LAR/SLC	2	475	950	
5 PEJ	Support meeting w/Becton Dickenson 3 days. Review commercialization.	LAR/SFO	1	1000	1000	
6 PEJ	Consultant's lab, Bozeman, MT 3 days. Review microbiology tests.	LAR/Bozeman	1	950	950	

E. TRAVEL		1. DOMESTIC				$ 11,150
		2. FOREIGN				
name	Purpose	Location	#trips	$ ea	total	
1 RWS	Project meetings, Soft Ray, Laramie 3 days. Discuss progress & plans	SLC/LAR	10	475	4750	
2 PEJ	Technical meeting on Cytometry 5 days. Attend technical papers.	LAR/SFO	1	1400	1400	
3 RWS	Consultant's lab, Bozeman, MT 3 days. Review microbiology tests	SLC/Bozeman	1	900	900	
4 PEJ	Present Paper on Cytometry 4days. Present research results.	LAR/BOS	1	1200	1200	
5 PEJ	Project meetings, Salt Lake City,UT 3 days. Discuss progress & plans.	LAR/SLC	2	475	950	
6 PEJ	Support meeting w/Becton Dickinson 3 days. . Review commercialization.	LAR/SFO	1	1000	1000	
7 PEJ	Consultant's lab, Bozeman, MT 3 days. Review microbiology tests.	LAR/Bozeman	1	950	950	

Airfare- coach: LAR/Bozeman, LAR/SFO, LAR/BOS, Private Vehicle-SLC/LAR, LAR/SLC, SLC/BOZ, BOZ/SLC, LAR/SLC. Lodging-actual, per diem- $35/day. Mileage- $0.31/mile. Cab/Bus Fare-actual. Receipt required for items $10 or over.

Materials and Supplies: Provide an itemized listing of materials and supplies. Provide quotations or other appropriate documentation to support any single material and supply item with an extended amount in excess of $5,000.

Materials:

1. MATERIALS AND SUPPLIES 1st YEAR		22200
1.1 Microscope fluorescence conversion modules Optical components $3,000. Power module $1,000, mounting attachments $1,000.	5000	
1.2 Improved TDI/CCD camera	2500	
1.3 Optics: collimators, focusing, reimaging, mirrors. 2ea optical collimators, 4ea focusing lenses, 4ea reimaging lenses,4ea first surface mirrors	2800	
1.4 Computer	2000	
1.5 Pump: SERINGE PUMP	400	
1.6 Laser: LED, gas, dye. 8ea LEDs, 1ea gas laser, 1ea dye laser.	2000	
1.7 Chemical/biological: Labeled antibodies $2,000, labeled beads $1,500.	3500	
1.8 Filters: Interference filters, $1,000 notch filters $1,000	2000	
1.9 IDL: Programming. Interactive data language program.	1500	
1.10 Bacteria: simulants	500	

1. MATERIALS AND SUPPLIES 2nd Year		$ 4,840
1.1 Chemical/biological: markers, dyes, beads	1,200	
1.2 Optics: filters, interference, notch, mirrors,	1,000	
1.3 Bacteria: biological simulant.	640	
1.4 Laser: Gas Laser	2,000	

Publication Costs/Documentation/Dissemination: Provide an explanation of items included in this amount and the per item cost (e.g., the estimated number of pages and the per page cost.)

No cost

Consultant Services: For each consultant, provide an agreement which includes the following information: (1) the services to be provided; (2) the period of performance and the consultant's availability; (3) the qualifications of the consultant to perform the work; and (4) the rate of pay (not to exceed $453 per day). (Consultants should be chosen using a competitive selection process; otherwise, sole source justifications should be maintained.)

3.CONSULTANT SERVICES 1st YEAR				$7,950
3.1 Douglas A. Christensen, Ph. D. (EE/Bio. Elec. Engr)	16hrs	$75/hr	1200	

	specific work in Bioengineering, Laser technology, review				
3.2	James J. Smith, Ph. D. (Microbiologist) Experimental Design and review	66hrs	$75/hr	4950	
3.3	Warrie Means, Ph. D. (Meat Scientist) testing meat samples and review	24hrs	$75/hr	1800	

3.CONSULTANT SERVICES 2nd YEAR					**$7,950**
3.1	Douglas A. Christensen, Ph. D. (EE/Bio. Elec. Engr) specific work in Bioengineering, Laser technology, review	12hrs	$75/hr	900	
3.2	James J. Smith, Ph. D. (Microbiologist) Experimental Design and review	66hrs	$75/hr	4950	
3.3	Warrie Means, Ph. D. (Meat Scientist) testing meat samples and review	28hrs	$75/hr	2100	

Each consultant will work not more than 6 hours in any day ($450) which does not exceed $453. The original proposal includes their letter of commitment and a resume which demonstrates their qualifications as a consult on this proposal.

Subawards: For each subaward agreement provide (1) the services to be provided, the names of individuals expected to perform the work, and the expected level of effort for each individual; (2) cost information broken out by cost category (salaries, fringe, travel.

Subaward to the University of Wyoming
Personnel:

A. SENIOR PERSONAL: PI/PD 1st YEAR		Period of performance	Funds
1 **Dr. Paul E. Johnson** Direct the subcontract activities	**PD**	3 months of activity over 1 year	0
2 **(To be hired)** Provide mechanical and electronic design for the Cytometer, controls, and tests of the cytometer system	**Engineer**	9 months of activity over 1 year	$21,016

B. OTHER PERSONNEL 1st YEAR		
3. GRADUATE STUDENT Assist the Engineer particularly with the testing of system	560 hours	$9,000
4. UNDERGRADUATE STUDENT Carry out work assigned and work with the engineer and graduate student	714hours	$5,000

G. OTHER DIRECT COSTS 1st YEAR			
1. MATERIALS AND SUPPLIES			$7,000
1.1 Mech .& Optical Shop (design & construction of cytometer, optics and controls)	280 hrs	$5,600	
1.2 Graduate student tuition (requirement of the University of Wyoming)		$1,400	

A. SENIOR PERSONAL: PI/PD 2nd YEAR			Period of performance	Funds
1	**Paul E. Johnson** Direct the subcontract activities	**PD**	3 months of activity over 1 year	0
2	**(To be hired)** Provide mechanical and electronic design for the Cytometer, controls, and tests of the cytometer system	**Engineer**	12 months	$28,020

B 4. UNDERGRADUATE STUDENT 2nd YEAR	714 hours	$5,000
Carry out work assigned and work with the engineer and graduate student		

G. OTHER DIRECT COSTS 2nd YEAR		
1.1 Mech .& Optical Shop (design & construction of cytometer, optics and controls)	280 hrs	$5,600

For Year 1 and Year 2, <u>Fringe Benefits:</u> FICA 7.65%; Unemployment 01.5%; workers comp., Health insurance, dental care, life insurance, eye glasses, other reasonable and necessary medical procedures; receipts required; The total for all these benefits may not exceed 28% of wages.

The total budgets for the subaward are shown in the first and second year budgets in the original proposal.

Indirect Costs: Provide a current indirect cost rate agreement negotiated by a Federal Agency. In the absence of a current negotiated rate agreement, provide an indirect cost proposal and the related financial statements (income statement and balance sheet) for the two most recently completed annual accounting fiscal periods. If an organization has not had financial activity for these periods, indirect cost proposals should be based on what activities the organization expects during the proposed award period. (See sample indirect cost proposal found at Appendix 6 of the Guide.)

Indirect Costs: 25% on total direct cost less subaward.
10% on subaward
7% Fee on Total direct cost plus Over head

In order to avoid delays in the review and processing of your proposal, please send the requested information within 15 days of the above date to:

To: National Science Foundation
Division of Contracts, Policy and Oversight
Cost Analysis and Audit Resolution Branch
4201 Wilson Boulevard, Room 475
Arlington, VA 22230
Cc: to Bruce Hamilton

Questions related to Items 1 and 2 should be directed to the Division of Grants and Agreements (Attn: Andrea Kline) at 703-306-1212. Questions related to Items 3 through 5 should be directed to the Cost Analysis and Audit Resolution Branch (Attn: Patricia Farrell) at 703-306-1244.

Status: U
Date: Fri, 01 Nov 2002 15:12:05 -0700
From: "Paul E. Johnson" <PJohnson@uwyo.edu>
Subject: FW: letter rpt #2
To: bales@mbj-law.com
Thread-Topic: letter rpt #2
Thread-Index: Ab/8vcHK0sa5GWipEdSErwCqAN2PpYUKawmQAAAbesA=
X-Message-Flag: Follow up
X-OriginalArrivalTime: 01 Nov 2002 22:12:05.0465 (UTC)
FILETIME=[B6429C90:01C281F3]

-----Original Message-----
From: Paul E. Johnson
Sent: Friday, November 01, 2002 3:09 PM
To: Paul E. Johnson
Subject: FW: letter rpt #2

-----Original Message-----
From: Richard W. Shorthill [mailto:shorth@eng.utah.edu]
Sent: Wednesday, August 02, 2000 2:13 PM
To: Bruce Hamilton; Paul E. Johnson
Subject: letter rpt #2

Bruce:

Attached is our planning document. Paul still plans to visit Becton Dickenson in about a month and go over our research plans as per the attachment. Our consultants are reviewing our planning document. We have obtained a sample of the "Jack-In -The-Box" Hamburger sample with E.coli 0157:H7 and our microbiologist will be working with it.

Dick

PS; Hope you can read the attachment.

Richard W. Shorthill, Ph. D.
University of Utah
Mechanical Engineering
50 S. Campus Drive
Salt Lake City, UT 84124-9208
UU Office E-mail: shorth@eng.utah.edu
UU Phone: 801-581-8623
UU FAX: 801-585-982

Home E-mail: srw4@uswest.net
Home Phone: 801-278-7042

Content-type: application/applefile; name=plan1.doc
Content-disposition: attachment; filename=plan1.doc
Content-description: plan1.doc

plan1.doc

Brief letter report #2:

STTR Phase II: "Early Detection and Identification of Individual Pathogenic Microorganisms in Food with a Flow Cytometer"

Planing outline

Work Breakdown for June 15th 2000 to June 15th 2001.

Task 0 Administrative Tasks (complete by August 15th)

- 0.1 Hire accountant. **Done**
- 0.2 Develop bookkeeping system. **Done**
- 0.3 Develop and implement time recording system. **Done**
- 0.4 Develop reporting and lab notebook system.
- 0.5 Develop budget reporting system.
- 0.6 Decide on project management software and purchase.
- 0.7 Rent office space. **Done**
- 0.8 Purchase computers. **Done**
- 0.9 Network computers.
- 0.10 Purchase and install computer software.
- 0.11 Develop company web site.
- 0.12 Issue press release.
- 0.13 Develop hiring plan.
- 0.14 Develop fringe benefit plan.
- 0.15 Purchase insurance.
- 0.16 Develop travel policy and forms.

Task 1 Review the Optimal Experimental Configuration for the Demonstration Prototype (complete by August 30)

- 1.1 Review w Warrie Means & Jim Smith USDA requirements for ground beef analysis.
- 1.2 Review w BD USDA requirements for ground beef analysis (August 18 meeting).
- 1.3 Review overall performance criteria (e.g. size, speed, Ease of use, sensitivity, volume throughput, Rate of false positives, detection efficiency).
- 1.4 Review proposed preliminary design and experiment design w consultants & Students
- 1.5 Produce revisions to preliminary designs
- 1.6 Refine WBS based on revision.

Task 2 Build Multi-color Demonstration Prototype (complete by December 15, 2000)

- 2.1 Build rectangular flow cell **Done**
- 2.2 Improve fabrication technique for flow cell **Background task**
- 2.3 Increase N.A. of optics **RWS review N.A. argument**
- 2.4 Incorporate faster CCD camera to permit faster flow
  - 2.4.1 Obtain camera specs from Electrim
  - 2.4.2 Decide on camera model & purchase
  - 2.4.3 Contract for TDI software drivers
  - 2.4.4 Contract to RSI for IDL software mods
  - 2.4.5 Modify SoftRay software for fast camera
  - 2.4.6 Modify SoftRay software for 2-colors
  - 2.4.7 Test SR software
- 2.5 Conversion of data acquisition software to faster C++ code. **(Break this down.)**
- 2.6 Addition of mirror/dichroic combination to divide the image of flow cell into two.
  - 2.6.1 Design preliminary test version of optics w CCD camera, but wo flow.
  - 2.6.2 Order parts.
  - 2.6.3 Construct apparatus on optical breadboard.
  - 2.6.4 Test apparatus.
  - 2.6.5 Integrate system w flow cell and two illumination sources

Plan 1

(LED or diode laser).

2.7 Develop and test LEDs as illumination sources (red and green). (Test with microscope and florescent beads.) **(Optional)**

- 2.7.1 Buy LEDs and optics.
- 2.7.2 Design and fabricate illumination system to be integrated with microscope.
- 2.7.3 Measure power of illumination sources using laser power meter.
- 2.7.4 Compare LED illumination sources with conventional low power laser using Olympus microscope.

Task 3 Perform Measurements using Florescent Spheres (December 15th on)

3.1 Determine which spheres to purchase & set up protocol for S/N tests and efficiency of detection tests

3.2 Purchase spheres

3.3 Run spheres through 1-color system for calibration, S/N analysis, and efficiency analysis

3.4 Repeat 1-color S/N analysis and efficiency analysis from Phase I using existing software and new C software **after January 1st.**

3.5 Determine protocol for S/N test to improve & optimize for best detection efficiency.

3.6 Execute protocol from previous step.

3.7 Modify design based on 3.6.

Task 4: Measurements w E. coli.

4.1 Modify lab for bio experiments, purchase necessary supplies, and determine lab safety and notetaking protocols. **Done**

4.2 Order and begin culturing E. coli. **Done**

4.3 Perform 1-color testing.

- 4.3.1 Determine labeling protocol **for 1-color measurements**.
- 4.3.2 Test protocol on O157:H7 and benign E. coli to determine scope of problems with cross-reactivity. (Use epifluorescent microscope.)
- 4.3.3 Make necessary corrections.
- 4.3.4 Label E. coli (benign, live) and count w microscope & flow cell for comparison.
- 4.3.5 Perform detection efficiency calculation.
- 4.3.6 Review.
- 4.3.5 Label E. coli (benign, dead) and count w microscope & flow cell for comparison.
- 4.3.6 Perform detection efficiency calculation.
- 4.3.7 Review.
- 4.3.8 Label O157:H7, dead and count (before or after killed?).
- 4.3.9 Perform detection efficiency calculation.
- 4.3.10 Determine protocol for mixed O157:H7/benign E. coli (all dead? Or just O157) mixed sample to determine cross-reactivity.
- 4.3.11 Count mixed samples to determine cross-reactivity (rate of false positives).
- 4.3.12 Review.
  - 4.3.12.1 Determine protocol for counting E. coli in ground beef spiked w O157 **after Jan.1.**
  - 4.3.12.2 Benign
  - 4.3.12.3 O157
  - 4.3.12.4 mixed E. coli
  - 4.3.12.5 Review
- 4.3.13 Count E. coli in ground beef spiked w O157
  - 4.3.13.1 Run sample in sterile ground beef with no E. coli
  - 4.3.13.2 with benign E. coli, unlabelled
  - 4.3.13.3 benign labeled
  - 4.3.13.4 O157, labeled
  - 4.3.13.5 Mixed, labeled
  - 4.3.13.6 Calculate efficiencies, false negatives, false positives

4.3.13.7 Review

4.4 Perform 2-color testing

4.5.1 Determine experimental design

4.5.2 Label E. coli (benign) and count w microscope & flow cell for comparison.

4.5.3 Perform detection efficiency calculation.

4.5.4 Review.

4.5.5 Label O157:H7 and count.

4.5.6 Perform detection efficiency calculation.

4.5.7 Determine protocol for E. coli mixed sample to determine cross-reactivity.

4.5.8 Count mixed samples to determine cross-reactivity (rate of false positives).

4.5.9 Review.

4.5.10 Determine protocol for counting E. coli in ground beef spiked w

4.5.10.1 O157:H7

4.5.10.2 Benign

4.5.10.3 mixed E. coli

4.5.11 Count E. coli in ground beef spiked w

4.5.11.1 O157:H7

4.5.11.2 Benign

4.5.11.3 Mixed

4.5.12 Review

High risks tasks:

1. **Build flow tube.**
2. **Label O157 and test vs. benign.**
3. **Separate pathogen from ground beef.**

EXHIBIT G

Kristin J. Bobbitt

From: Paul E. Johnson
Sent: Friday, July 21, 2000 11:37 AM
To: 'jim00@in-tch.com'; 'shorth@eng.utah.edu'; Warrie J. Means; Kristin J. Bobbitt; 'sundance@uwyo.edu'; 'lunds2@uwyo.edu'
Subject: Work Breakdown


WBS.htm

SoftRay team members,

I have attached (in html) a first-draft work breakdown for the first year of our NSF Phase II. Please review it and give me modifications/comments, as well as indicate to me where you would like to participate. I will attempt to put in dates in the next version. This WBS will serve as the basis for weekly task review meetings and biweekly email progress reports to the NSF.

Please note that each line should be no longer than two weeks in time. Otherwise it needs to be broken into subtasks. Some of the tasks that I have listed here might not be realistically completed in two weeks.

You will need to refer to the original proposal in order to adequately interpret this WBS.

I would like to have this plan finished within two weeks in order to begin interaction with Becton Dickinson. I meet with BD in San Jose on August 18th.

I can't tell you how excited I am that this project is now underway.

Thank you,

Paul

Work Breakdown for June 15th 2000 to June 15th 2001.

Highlighted tasks are to be started after January 1, 2001. Any single line that will take over two weeks should be broken into subtasks, each of which should take less than two weeks.

Task 0 Administrative Tasks (complete by August 15th)

0.1 Hire accountant. Done
0.2 Develop bookkeeping system.
0.3 Develop and implement time recording system.
0.4 Develop reporting and lab notebook system.
0.5 Develop budget reporting system.
0.6 Decide on project management software and purchase.
0.7 Rent office space.
0.8 Purchase computers. Done
0.9 Network computers.
0.10 Purchase and install computer software.
0.11 Develop company web site.
0.12 Issue press release.
0.13 Develop hiring plan.
0.14 Develop fringe benefit plan.
0.15 Purchase insurance.
0.16 Develop travel policy and forms.

Task 1 Review the Optimal Experimental Configuration for the Demonstration Prototype (complete by August 30)

1.1 Review w BD USDA requirements for hamburger analysis.
1.2 Review w Warrie Means & Jim Smith USDA require-
Ments for hamburger analysis.
1.3 Review overall performance criteria (e.g. size, speed,
Ease of use, sensitivity, volume throughput,
Rate of false positives, detection efficiency).
1.4 Review proposed preliminary design w consultants &
Students
1.5 Produce revisions to preliminary design
1.6 Refine WBS based on revision.

Task 2 Build Multi-color Demonstration Prototype (complete by December 15, 2000)

2.1 Build rectangular flow cell Done
2.2 Improve fabrication technique for flow cell Background task
2.3 Increase N.A. of optics RWS review N.A. argument
2.4 Incorporate faster CCD camer to permit faster flow
2.4.1 Obtain camera specs from Electrim
2.4.2 Decide on camera model & purchase
2.4.3 Contract for TDI software drivers
2.4.4 Contract to RSI for IDL software mods
2.4.5 Modify SoftRay software for fast camera
2.4.6 Modify SoftRay software for 2-colors
2.4.7 Test SR software
2.5 Conversion of data acquisition software to faster C++ code. **(Break this down.)**
2.6 Addition of mirror/dichroic combination to divide the image
of flow cell into two.
2.6.1 Design preliminary test version of optics w CCD camera,
but wo flow.
2.6.2 Order parts.
2.6.3 Construct apparaturs on optical breadboard.
2.6.4 Test apparatus.
2.6.5 Integrate system w flow cell and two illumination sources
(LED or diode laser).

2.7 Develop and test LEDs as illumination sources (red and green). (Test with microscope and florescent beads.) **(Optional)**
- 2.7.1 Buy LEDs and optics.
- 2.7.2 Design and fabricate illumination system to be integrated with microscope.
- 2.7.3 Measure power of illumination sources using laser power meter.
- 2.7.4 Compare LED illumination sources with conventional low power laser using Olympus microscope.

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3.1 Determine which spheres to purchase & set up protocol for S/N tests and efficiency of detection tests
3.2 Purchase spheres
3.3 Run spheres through 1-color system for calibration, S/N analysis, and efficiency analysis
3.4 Repeat 1-color S/N analysis and efficiency analysis from Phase I using existing software and new C software after January 1st.
3.5 Determine protocol for S/N test to improve & optimize for best detection efficiency.
3.6 Execute protocol from previous step.
3.7 Modify design based on 3.6.

Task 4: Measurements w E. coli.

4.1 Modify lab for bio experiments, purchase necessary supplies, and determine lab safety and notetaking protocols. Done
4.2 Order and begin culturing E. coli. Done
4.3 Perform 1-color testing.
- 4.3.1 Determine labeling protocol **for 1-color measurements.**
- 4.3.2 Label E. coli (benign) and count w microscope & flow cell for comparison.
- 4.3.3 Perform detection efficiency calculation.
- 4.3.4 Review.
- 4.3.5 Label O157:H7 and count.
- 4.3.6 Perform detection efficiency calculation.
- 4.3.7 Determine protocol for mixed O157:H7/benign E. coli mixed sample to determine cross-reactivity.
- 4.3.8 Count mixed samples to determine cross-reactivity (rate of false positives).
- 4.3.9 Review.
- 4.3.10 Determine protocol for counting E. coli in hamburger spiked w after Jan. 1
  - 4.3.10.1 O157:H7
  - 4.3.11 benign
  - 4.3.12 mixed E. coli
- 4.3.11 Count E. coli in hamburger spiked w
  - 4.3.11.1 O157:H7
  - 4.3.11.2 Benign
  - 4.3.11.3 Mixed
- 4.3.12 Review

4.4. Perform 2-color testing
- 4.4.2 Label E. coli (benign) and count w microscope & flow cell for comparison.
- 4.4.3 Perform detection efficiency calculation.
- 4.4.4 Review.
- 4.4.5 Label O157:H7 and count.
- 4.4.6 Perform detection efficiency calculation.
- 4.4.7 Determine protocol for mixed O157:H7/benign E. coli mixed sample to determine cross-reactivity.
- 4.4.8 Count mixed samples to determine cross-reactivity (rate of false positives).
- 4.4.9 Review.
- 4.4.10 Determine protocol for counting E. coli in hamburger spiked w
  - 4.4.10.1 O157:H7

4.4.11 benign
4.4.12 mixed E. coli
4.4.11Count E. coli in hamburger spiked w
4.4.11.1 O157:H7
4.4.11.2 Benign
4.4.11.3 Mixed
4.4.12 Review

EXHIBIT H

From Notebook 2 Paul Johnson

Sept 4 Holiday

Sept 5
met w Julie Kellogg regarding use of LEDs in flow

met w Mike Lund re fixing leak in flow-cell

Sept 6
met w Mike Lund

Sept 7
Weekly telecon: w only Dick & Paul } 2 hours

~~Aug~~ Revised WBS
Wrote up minutes
Worked on expense account

Telecon w Winslow
Winslow suggested not signing any agreement w BD that gives them first right of refusal until they have made a monetary committment

Sept. 21

Telecon: Dick, Paul, Jerome, Michael, Kristin

1. Jerome: server will be up soon with TP is up. Used for packing.
2. Michael - web page antibody staining tested on Saturday CCD dark current

A.I. - camera manual

3. Geologist being hired
4. How cell is being tested on Sat. with "breaking barrier"
5. Website nearly complete

Completed Safety and safety meeting w Michael. Need Key to slot

A.I.

Emailed changing domain for www.sofbeg.inc to www.concentric.com

Met w Julie re: LED testing.

Called Jim Smith:

A.I. Before we choose labels do absorption spectrophotometry of beef washings.

Sept. 22

Arranged with www.uconference.com for conferences calls at $25 per call ($99/month for one call a week plus $25 for each additional. Prepared by Michael and

met w Michael

Read Cytomely article from July 1, 2000 on triple label for viability + antibody

Sept 25

Prepared for telecon.

Telecon w/ BD Baltimore (Tom Wannlund, Jim Smith, Lunch, + Mondar Nagar)

See minutes in 3-ring binder.

Sent out minutes - of meeting.
Met with Harvey re 1890.
Telecon w Krissy Marion + Susan Gpetka (BD)

~~Fact~~ Recommendation to make agreement with Univ. of Utah.
Syn stf on tech transfer office

AI < Copy of letter to ~~Univ. of~~ Shorthill

AI < Background intellectual property in final agreement w UW.

AI < Send draft of Shorthill agreement to BD.

Posted job ads in Rote Ag + Animal Sci.

Sept 26

Met w Julie re LEDs.

Worked on revised update + sent to Bob Hoffman. (Updated w his comments). See notebook copy.

Sept 27th

Interviewed Amanda for job. (in Bio.)

Faxed CV to Deb (Amanda)

Oct 25

Met w Amanda. She is looking at installing eye wash & swapping lab towels.

Met w Michael

Met w Jerome and discussed how to use longs remaining in C++ Account.

Worked on travel and expenses.

Met w Kristen. Will study money market today. W. Mark Mader to work on discrepant

Worked on time sheet. 2 1125 bill.

Talked to Curtis. Work order submitted for card lock in pathogen room.

Oct 26

Weekly Conference

Amanda, Jerome, Julie, & Michael (w me)

New action items

1. Talk to Warrie re: Confocal visit. Paul
2. Build rectangular LED beam optics. Julie
3. Test LED on oscope w beads. Julie
4. Ask Warrie about Confocal tour. Paul
5. Spend 2-3 hours on C++ software. Jerome & Pu
6. Move equipment. Everyone.
7. Ask Shirl about BAM, AOAC manual. Paul

Met w Julie. Gave her copy of Don Klopstein's LED main page.

- http://www.mistry.com/~don/ledx.html
- See also ledmuseum.home.att.net

Suggested that see try semi-cylindrical lens for rectangular (~2mm x 200μm) beam.

Nov 14

Interviewed 2 hour three.

Paul E. Johnson

From: Paul E. Johnson
Sent: Tuesday, November 14, 2000 3:34 PM
To: 'julee@uwyo.edu'
Cc: 'shorth@eng.utah.edu'

Julie,

I talked to a physicist at Becton Dickinson. He said that they've actually been able to get more intensity out of the blue Nichia diodes running between 30 and 40 ma (although they might not run that hard forever).

Isn't your experience that the blue diodes fatigued at 30 ma like the green ones?

Bob suggested that the flat packs will probably dissipate heat better than the lensed diodes, so that we should be able to drive them harder.

Paul

Nov 15 Spent some time in lab helping reorganize.
2 hours on USDA proposal.

Nov 16 SR Meeting. (see A-T list). Worked on USDA proposal
Worked on swipe task for lab.

Nov 17 Worked with Julie on setting up CCD w/ microscope. Electrical running on scope doesn't work.

Talked to Amanda re antibodies.

Feb. 20th

Dennis + I talked to Kussy + Hoel re: contract. It looks like no up front money, but we will try to get foreign patent costs.

Amanda has scoped out larger centrifuge.

Jon Wannlund has recommended a possible source of mono polyclonals.

Email exchange: Debra is testing C++ bicount code.

Faxed 2-page LED disclosure to David.
Met w Amanda

2/21st

Emailed Jon W. Suggested that time is too short to do polyclonals.
Talked to Amanda re antibody selection.
Letter of Engagement sent from UW to Jennifer.
Worked w/ Julie on LED/microscope

2/22nd

SR Meeting
~~Antibody~~
Email from Swanson re Edgewood.
Tried to reach John Pope re: contract UW / Blue Sky

Feb 23

Met and worked w Julie on LEDs
Met w Amanda

3/26
Met w Amanda re 0.4157-447
medium.
email + tky w Jerome + Julie
re LEDs

3/27
email + tky w Kristy + Marion
re contract
met w Amanda

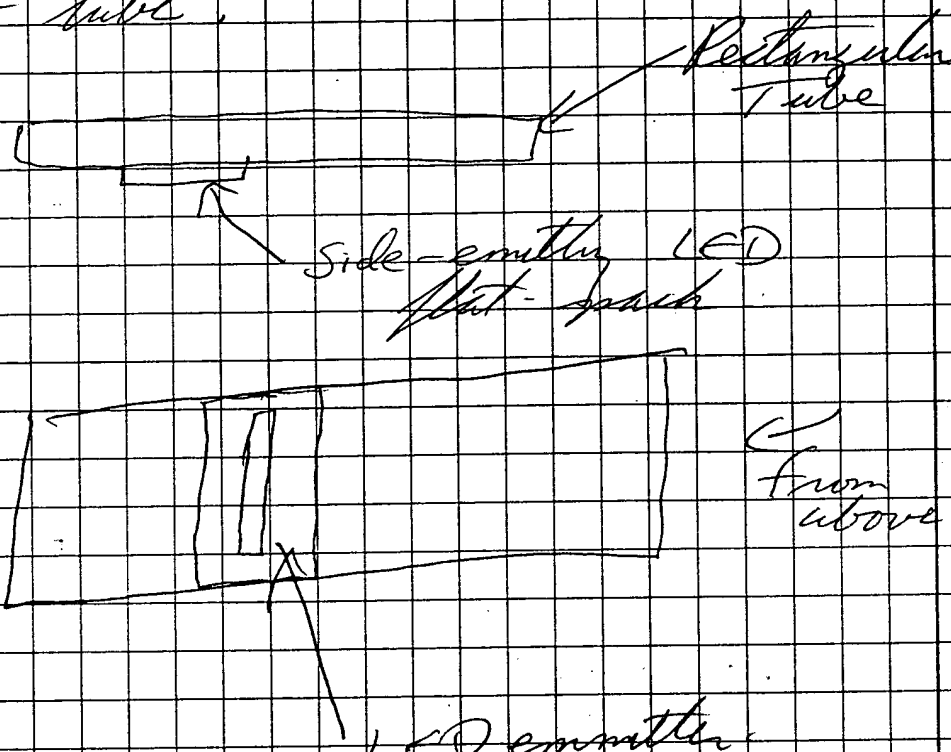
3/28
negotiation mtg. w Lanzilotti 1 hour
Met w Amanda
sent email to D. Cook re: negotiations

3/29 source of blue LEDs: www.hosfelt.com/leds/led1... 1 hour
Worked on Travel + time
Lanzilotti emailed Dem + Roger re:
negotiations

3/30
Met w Jerome
Time Sheets

3/30

My ideas for LED illumination of Floor Tube.



can glue (contact cement) more than 1 LED to Tube to achieve highest throughput.

I asked Jerome to order R-PE beads to illuminate with blue and/or green LEDs.

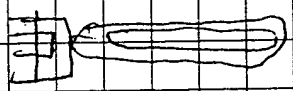
4/2/01
met w Kristen re size checks.

4/3/01
met w Jerome re LED illumination
met w Amanda

4/4/01
met w Jerome re LED illumination

4/5/01
Soft Ray mtg - 2 hours

4/6/01
met w Amanda
called Hoffman / no answer
called Jim Smith
worry about micelles absorbing label → put detergent into ground beef instead
get batteries for cross-reading from camera room



LED contacting battery

Paul E. Johnson

From: Paul E. Johnson
Sent: Friday, April 13, 2001 12:37 PM
To: 'shorth@eng.utah.edu'; Asprey, Bill; Asprey, William; Kellogg, Julie; Kristin Bobbitt; lunds2 @uwyo.edu; sundance@uwyo.edu; Votaw, Amanda; Zhao, Dehua
Subject: New Ideas!

Everyone,

I just talked to Bob Hoffmann at BD and gave him a verbal update on our progress. He made some terrific suggestions:

1. Illumination Sources: He suggested that we think about illuminating with laser illumination run through a multi-mode fiber to make it non-coherent. Even better-- LumiLeds makes LEDs that put out 100mW!!! They have emitting regions that are 1mm x 1mm. He suggests collimating this light with Kohler (with an umlaut) optics, that are used in microscopy to collimate light as well as to make the beam uniform. Then we could illuminate from above, at an angle, onto a 2mm x 1mm patch, or whatever, uniformly. Illumination from above through 100 microns of material would also alleviate absorption by the medium. Hoffman is sending a 505 nm LED for us to evaluate. He suggests replacing our microscope illuminator with this to test it.

2. Labels: If we have to illuminate in the red, because of solution opacity, Bob suggests APC, CY5, or one of the Molecular Probes Alexa dyes. He wanted to know whether our meat solution appears red, and how cloudy it appears?

3. Beads: If we want to experiment with bicolored beads for our 2-color measurements, he suggested talking to Ken Davis, who just happens to be developing two colored beads (based on attaching antibody to the beads, labeled with two different labels).

4. Detergent: If we need to add detergent to our disgusting beef cocktail, to keep miscles that absorb dye from being detected as bacteria, he suggests talking to Ken about the possible adverse effects that detergent will have on labels. Some labels can bind to detergent, others can react in other adverse ways.

An enormously profitable discussion!

Paul

EXHIBIT I

NAME	LOCKER/DESK NO.	COURSE & SECTION NO.

4/7/01

1. Telecon w Lysogyte. They appear to have IMS technology in patent.

2 Andy Seneff (Natick) telecon.
mentioned Terkegaard + Kerry antibody
(too expensive)
Biosensors

They are working on separation + recovery from food. CRADA!!
Filter Ceazr!!
Could be funded from Vetcom.

Demo in Sept.?

Talk to Janet.Tensen@sbccom.apgea.army.mil re water

Interested in rapid diagnostics for food. Easy to use
Field studies. Portable

Hoo

Book 3 P.T.

SIGNATURE	DATE	WITNESS/TA	DATE
	4/4/01		

EXP. NUMBER	EXPERIMENT/SUBJECT		
NAME		LOCKER/DESK NO.	COURSE & SECTION NO.

4/17/01

Time sheet.

SIGNATURE	DATE	WITNESS/TA	DATE
[signature]	4/17/01		

1. Bioimage install.txt (in Dan) describes IDL environment and installation routines.

2. Bioimage.pro data acquisition. compile @test
set TDI ~~set~~ 900.000 (corresponds to pump rate of 15 w/ ~~Dell~~ HP) (3 mL?)

3. PSP3.pro counting
logfile.txt

Bioimage.pro file preference startup
set working directory to ✡ c:\dan for sub-software (.pro files)

✡ look at bioimage install.txt ✡

stat analysis in c:\0105

anum3-8 (corrected.pro - iterated stats
anum3-8... other one does one ~~stat iteration~~

A. Bioimage 225000 is probably minimum exposure on Dell (pump rate of 60.)?

B. TDIfirst2.exe (source code in Public) ⇒ under TDI Source ⇒ dan never tried on null sample
set exposure 30 is 3.0 (use it) 4 spot age (Enter?)

C. Asprey's password is blacktdog

D. anum3-8... the short folder names so that they won't map.

compile
command "psp4sp"

✡ ~~stop~~ ✡
analysis goes into data.dat
read with excel

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#coauto
#coduto
#coname
} outputs from auto iteration version
multi - it doesn't require stop!
cut & paste coauto + coname
To import to excel
1st columns are counts, 2nd overlap.
image counts are cumulative!!

Dehuu's software accumulates. logfile.out goes into Dan tmat w 'y' for xcel

only need 30 images from runs

LEDs run 350ma couple minutes
cut output drops over time
20% drop in output from 525 ma

2mm spot size w LED

need to use both sets of lens pairs:
one for objective one for LED

Nile red is 10^{-10} out of bottle
start 1/1000 dilution w BPW

magnetic stir for 30 minutes
(4 on stir plate (visible vortex))
sonicate for 2 minutes.

SIGNATURE | DATE 11/11/06 | WITNESS/TA | DATE

August 22

Worked all morning on expenses / France.
met w Amanda
Worked several hours w Ryan
Greg Jackson called re renewing insurance policy. I asked Kristin to take care of this.

Aug 28
met w Amanda.
Worked w Ryan
Lab meeting.

Aug 29
Interviewed Jean Jewell.
met w Dennis to get Stock shares.
Worked w Ryan
Signed up for Venture West Conf. + SBIR CS Dakota reimbursement
Dropped sheet by David Tanguilli w Cap. Table & Stock shares.
Emailed Eugenia re phrotegy route.

Aug. 30
mailed RWS LED and K-1.
Emailed Debra + Jerome re access to lab computer.
Rcvd email re: new round of stunted beef.

SIGNATURE	DATE	WITNESS/TA	DATE
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NAME	LOCKER/DESK NO.	COURSE & SECTION NO.

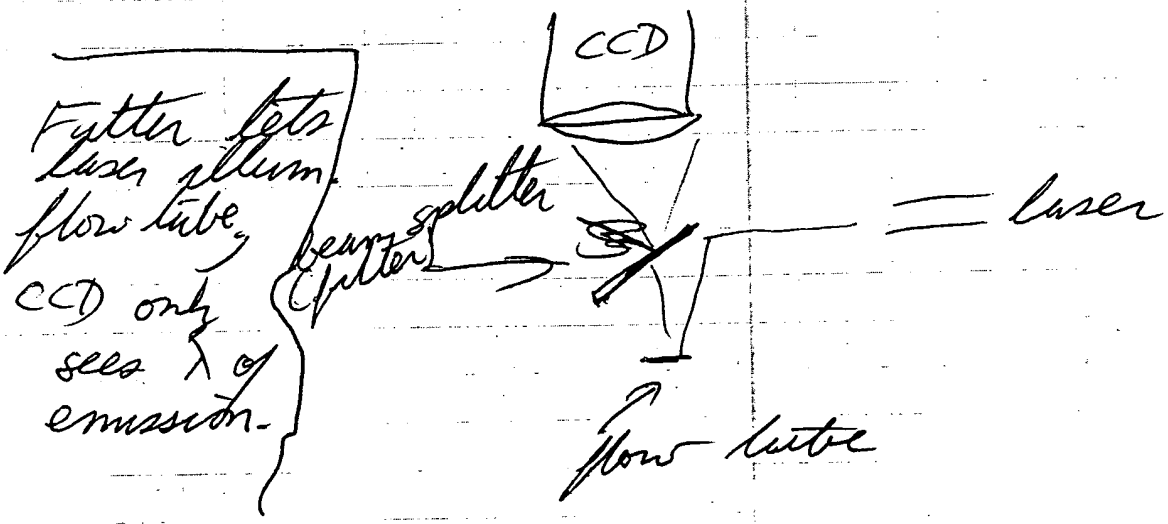
Worked on reports for BD.

August 31

Submitted revised ad for Boomerang-
Submitted (+ worked on) revised new
BD reports.

Sept. 4
called + sent info to Dan
at Equinox re: LED portion of
project.
met w Amanda.

Sept 5
Talked to John Miller about
building beam splitter



SIGNATURE	DATE	WITNESS/TA	DATE
	9/14/06		

Sept 13.

Lab mtg.

Mtg w Amanda.

Sent contract mods to Tussy/BD. (2 emails).

?

Sept 14

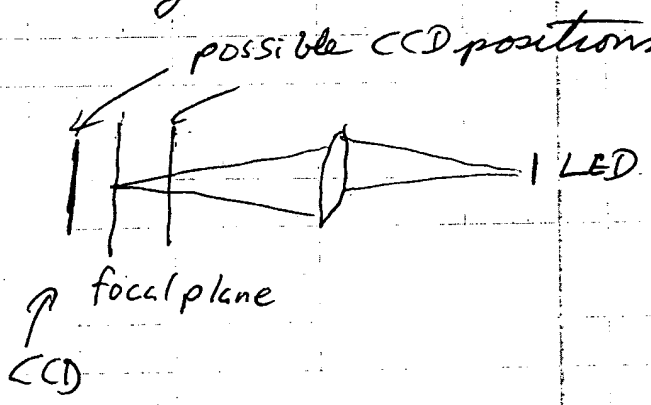
met w Amanda
worked on LED setup

Sept 17

Sent out signed CRADA.

Work on time reports.

Tests (continued) on LED setup, works great!



±10% uniform over 2mm × 2mm field.

NAME	LOCKER/DESK NO.	COURSE & SECTION NO.

Paul E. Johnson

From: Paul E. Johnson
Sent: Monday, September 17, 2001 12:19 PM
To: Amanda S. Votaw

Amanda,

I tried some more quantitative experiments with my crude LED setup in the lab. The result, after 20 minutes of experimenting, is that it is straightforward, if not trivial, to produce a square beam, 2mm across, that is within 10% of being uniform. I projected the LED beam onto the bare CCD and imaged it, after turning the LED down so far as to be almost off and turning the CCD exposure time down to 1 millisecond. I then produced row plots from the CCD that are pretty uniform. In order to get a large enough uniform beam, I just moved the CCD until the image of the LED was so far out of focus that it produced a uniform beam about 2-3 mm across.

After lunch I'll call LumiLeds and see if they are now shipping blue and cyan leds. If so, I'll order some leds and optics than are less humongous than what I'm using now. After these come in I'll have the machine shop put everything together in a nice aluminum or brass package.

Obviously, if Lumileds is shipping blue/cyan leds, we can use blue-illuminated dyes, such as FITC. In the short term, we can even use FITC.

Not bad for a morning's work, and a lot more fun than bead counting. Maybe I should quit for the day while I'm ahead.

Paul

Used Olive to try out possible lenses for LED illumination.

Try part # 32490 Edmund.
~~25~~ 25.00 mm DCX ef 25.00
at a distance ca. 30.0 mm
from LED.

Need 532 nm & 488 nm 10nm FWHM interference filters.

LEDs $8.00 ea/min of 50 from Future electronics

SIGNATURE	DATE	WITNESS/TA	DATE
[signature]	11/11/02		

NAME	LOCKER/DESK NO.	COURSE & SECTION NO.

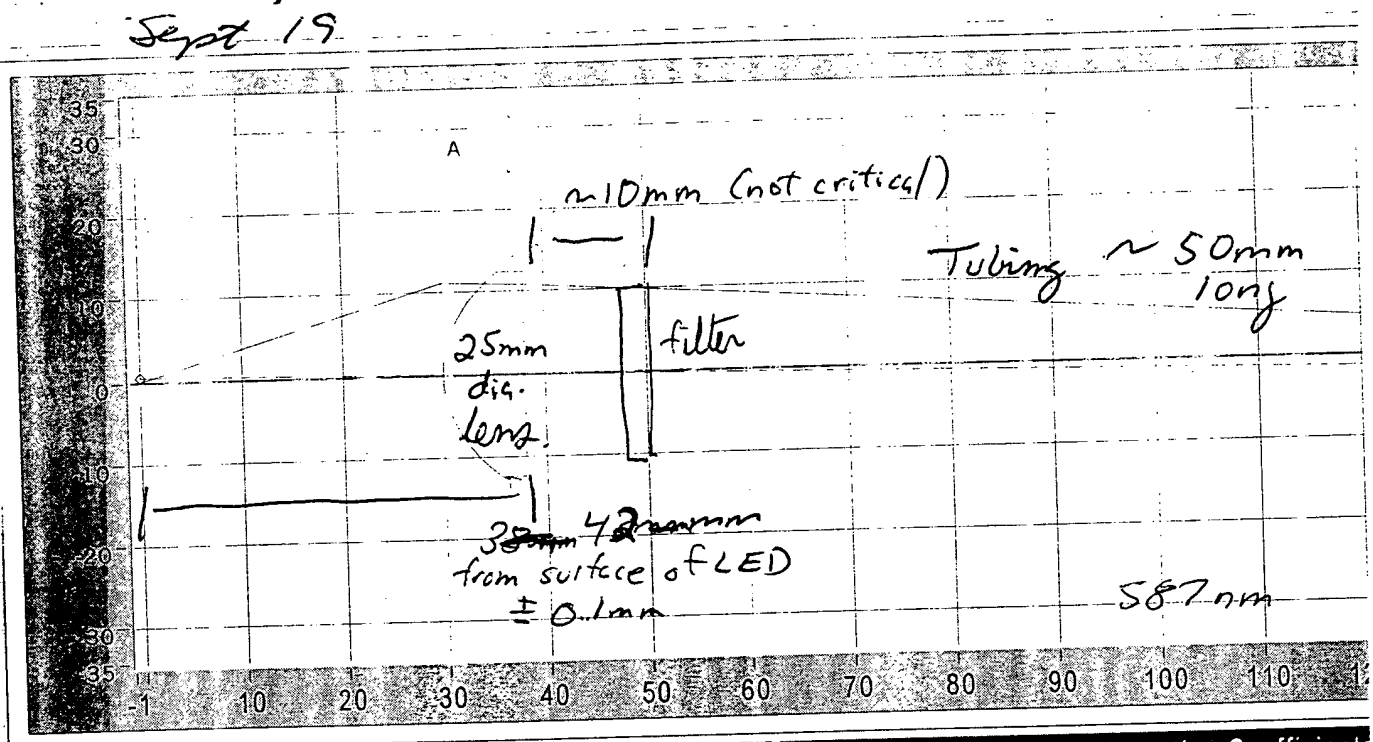
Sept 18

Met w Steve/Shops re data system box.
Met w Gus/Shops re illumination system box.

Tried out older square-board LED. it is green. Account# 4140712 49

Lumileds uses same "near optics" or collimated + non-collimated optics.

Sept 19



Paraxial Data

Surface	Z	Y marg.	Y chief	Radius
Object	0.000	0.000	0.500	inf.
1	30.000	11.250	0.000	16.820
2	38.000	10.891	-0.080	inf.
Image	182.951	0.000	-2.496	inf.

Seidel Aberration Coefficients

Lens	Sph.	Coma	Astig.	P
45098	4.9288	0.0780	0.0014	0
Totals	4.929	0.078	0.001	(

24/36

SIGNATURE	DATE	WITNESS/TA	DATE

Sept 19

met w Amanda

Questions on optics

1. How big is LED emission surface? 1mm x 1mm

2. How far is LED surface from mounting surface? measure

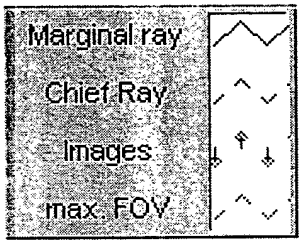
3. f/# of LED emission? 90°

4. Is using a DCX lens equivalent to a PCX in terms of mechanical design. No

5. 2-x mount?

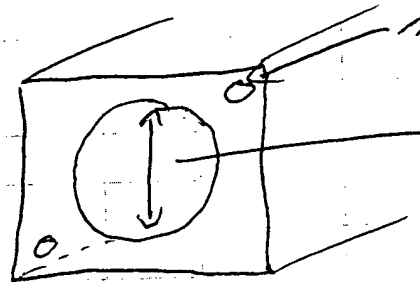
OLIVE 1.0.
see previous page

High Chiva
Systems
Tucson AZ
info@highchiva.c
www.highchiva.com



DATA	
Object size	0.50
Image size	-2.50
pMag	-4.99
parx. focus	144.95
system len.	182.95
working F#	6.65
Stop Radius	11.25

LED illuminator



On other end mount for 1" filter.

Spent all day on optical design for LED illuminator ≡ requisite IP w BD (mtg. w Dennis Cook, telecon w Noel, Kristy, & Bob Hoffman).

Gave machining job to Gus. Est. time to completion = 1 week.

Could go w aspheric condenser, such as Edmund L43-484.

- Need lens mount!
- Order new broader filters!!
- X-2 mount.
- Order LEDs.

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	LOCKER/DESK NO.	COURSE & SECTION NO.

Sept. 20

Met w Amanda.
Softray mfg.
Talked to John ⇒ tomorrow will be completion of reflective optics. Can do imaging tube next week.
Submitted ad to Boomerang for Engineer / Tech.

Sept 21

Mark Walton process consultant.

Looked at www.omegafilters.com
order green LUMILEDS
test w 488nm interference filter
but use omega filters
(from curvomatic) for FITC,
Alexa 488, R-PE.

Consider using aspheric condenser lens to collect more light. Problem w combo of f/# of collecting optics, magnification, & working distance. To keep mag. the same and go to faster optics, working distance must decrease.

Sept. 24

met w Amanda
worked on USGS proposal 2 hours
worked on camera lens design
(mocked up in lab) 2 hours

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[redacted]

Oct 1, 2001

Met w Gus.

Preliminary try-out of illumination optics. It works. Next step is to try even illumination test.

+DL0157 (SUbass)

apple 314 (Bios)

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[redacted] | LOCKER/DESK NO. | COURSE & SECTION NO.

add another 1cm to detector (farther than 1cm)

ledtest3.gif — 9cm from end of illuminator to end of camera
.2 amp thru led
1 ms intg. time
r. ±10% over 2mm

---

ledtest4.gif — ~~Room~~ 11cm | 1ms | 1ms
r ±10% over 2mm

---

ledtest5.gif — 5cm
r ±10% {spropfle need more square!!}

7.5cm in focus

ledtest6.gif

ledtest7.gif — 6cm in focus!

ledtest8.gif — seen again WONDERFUL!!

led bak.gif — background image!

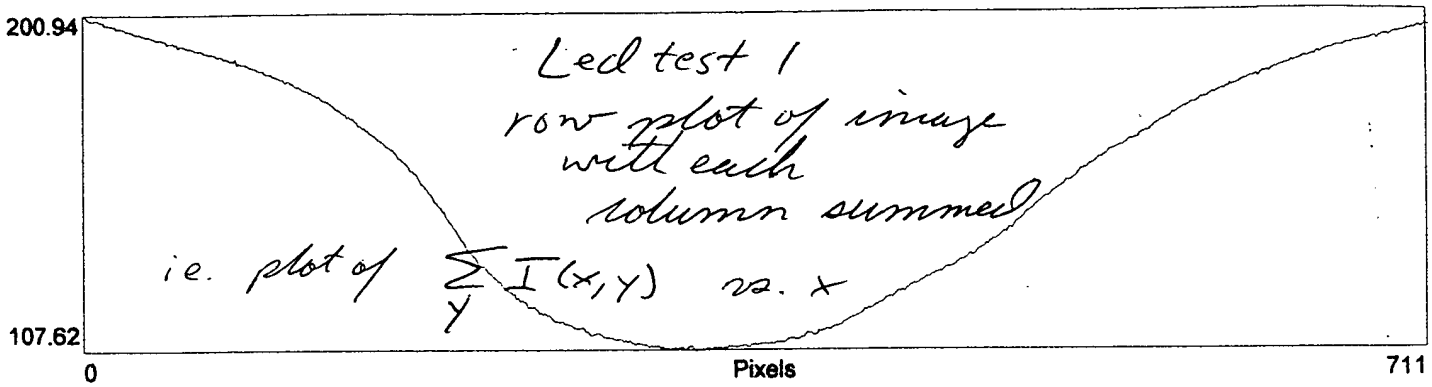
Power ns — .35 amps — 2.02 no filter ~.4 amps
.13 W 532 nm filter
(interference)

green? LED

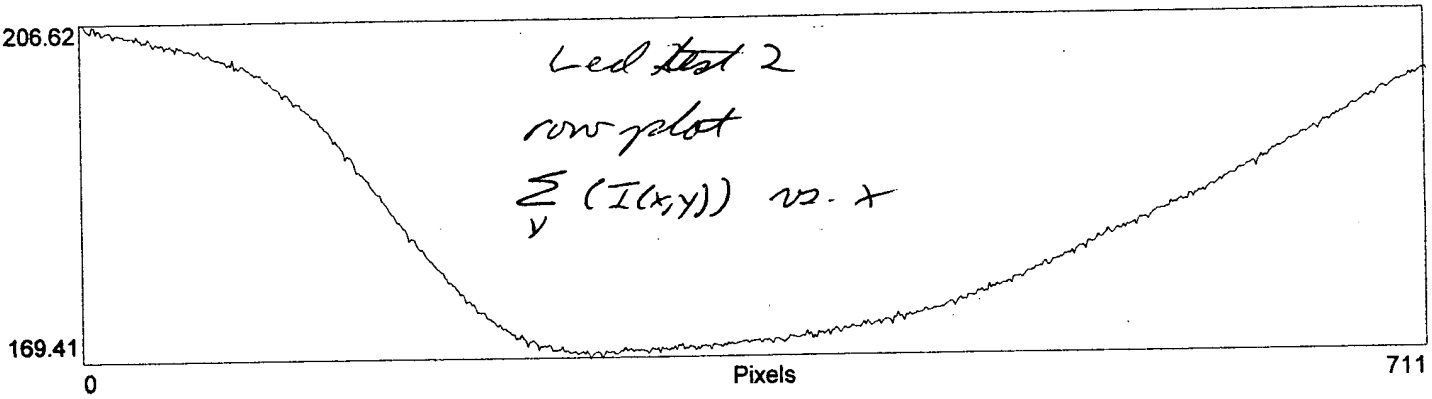
3.42 at ~.75 amps no filter

Low power due to slow f/# of optics.
f/2 system, need ~f/0.7 system.
Losing 90% of light!!!

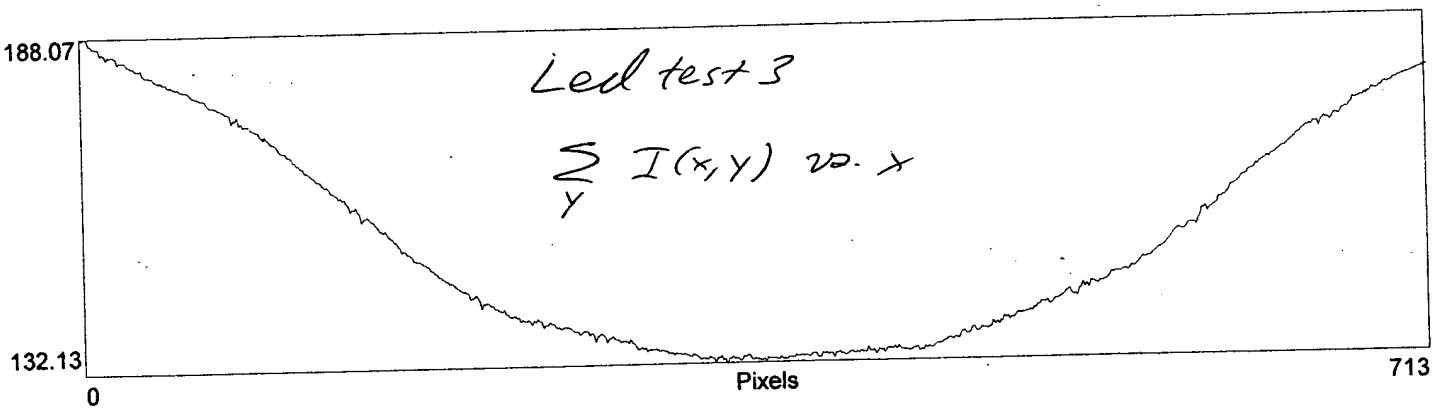
SIGNATURE	DATE	WITNESS/TA	DATE
[signature]	11/16/06		



Led test 1
row plot of image with each column summed
ie. plot of $\sum_y I(x,y)$ vs. x

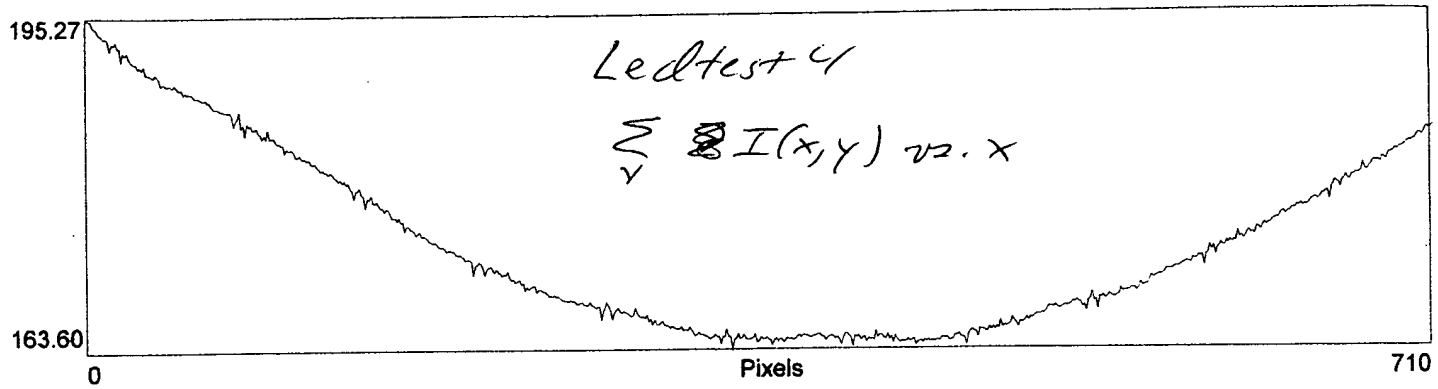


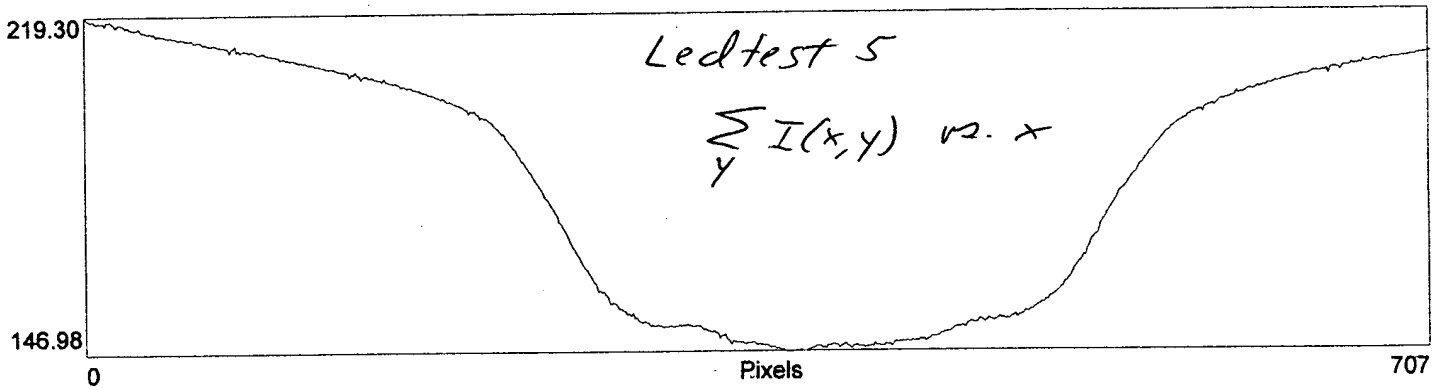
Led ~~test~~ 2
row plot
$\sum_y (I(x,y))$ vs. x

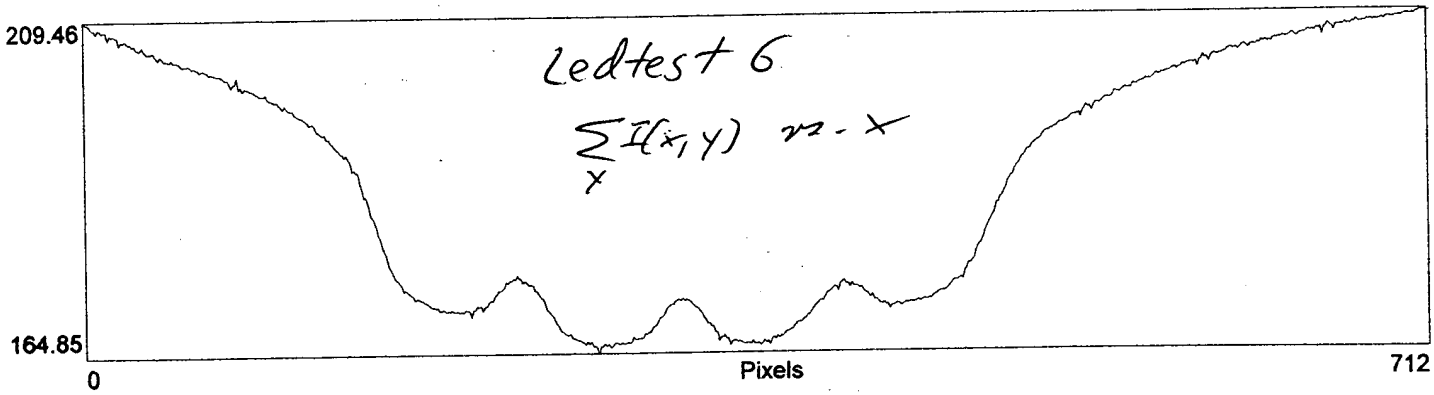


Led test 3
$\sum_y I(x,y)$ vs. x

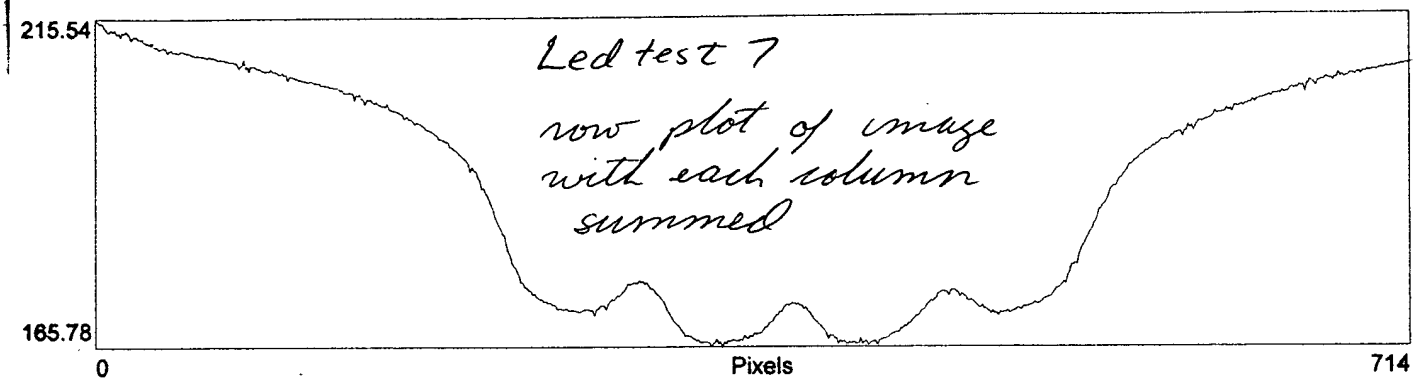
SIGNATURE	DATE	WITNESS/TA	DATE
	20/11/01		

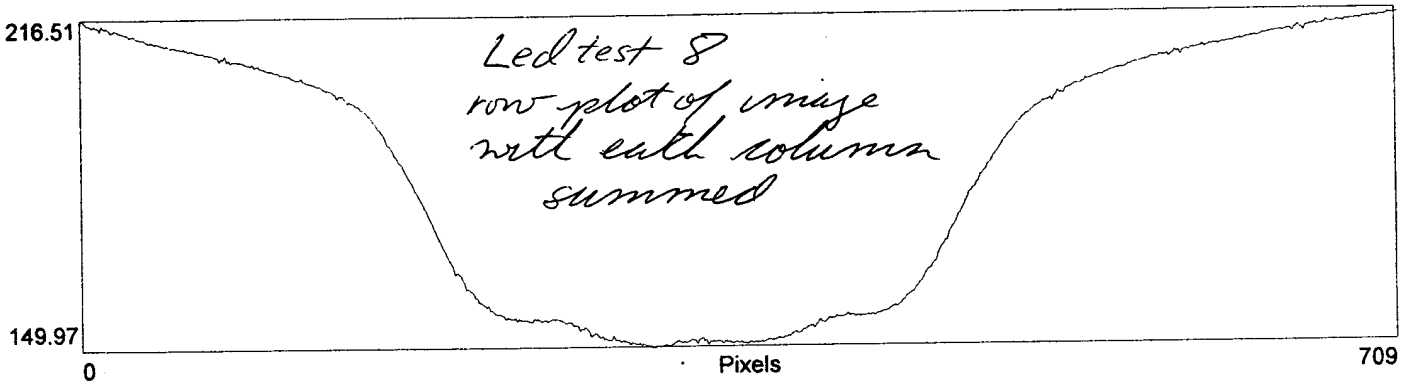






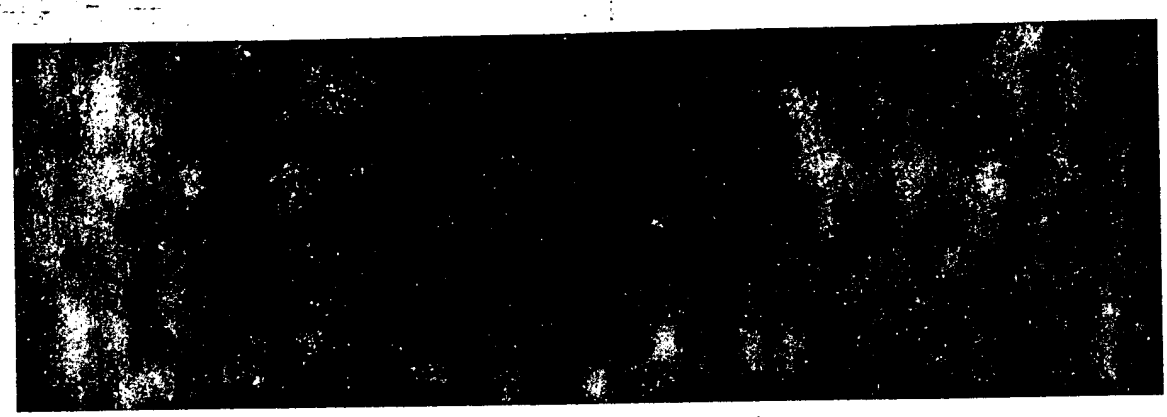
SIGNATURE	DATE	WITNESS/TA	DATE
	11/16/01		





Led test 5.sif

SIGNATURE	DATE	WITNESS/TA	DATE
	11/11/01		



Led test 6.8.4

Oct. 2
sent email to Dick re LED system

Oct. 3 ordered f/0.5 lens from Edmund.

Oct 4 Lab meeting.
asked Dan to modify LED illuminator for ~~LED~~ f/0.5 lens.
Played w f/0.5 lens.
Dick is sending x-y mover.

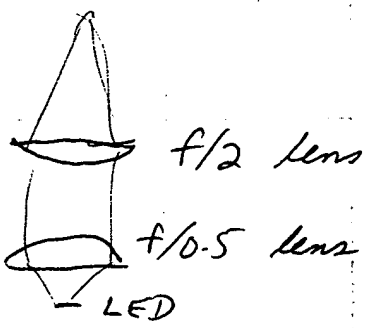
Oct 5
corrected illuminator for correct
FL.
Called Alison's rep. Heidi Allen
Worked on fusing stomacher.

Oct 7
Played with illuminator

SIGNATURE	DATE	WITNESS/TA	DATE
	11/11/05		

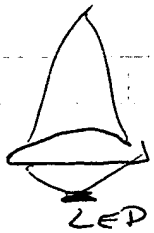
Oct. 8

Last week's
Current version of illuminator:



eff. focal length too short

Current version:



f/0.5 lens

350 ma ⇒ 5 mW
500 ma ⇒ 6 mW
} too small increase in speed from f/2 to f/0.5

Try new Cyan LED. Is green LED worn out?
Clean lens.
New detector?

Met w Allison 1 hour.

Played with optics

- Talked to Leo Weitzmann re proposals + SpinCon Sampler (N.d West Research Institute). www.mriresearch.org/ltd/spincon.html

SIGNATURE	DATE	WITNESS/TA	DATE
[signature]	11/11/01		

Oct 9

email letter from Krissy re BD collab.
not good

Deposit check (Finegra) to SR checking.

Oct 11

Lab mtg w Allison + Tony.

Investigate filters

Worked on proposal 2 hours.
USGS

Oct 12

Talked to Lee Sylvers. (claims in Rockville MD) sent CDA.

A Received filter specs from Ellen Fegner omega.

Worked on USGS proposal 4 hours.

Oct 14 ~~Order~~

Ordered filters from Edmund.
650 nm + 500 nm 80mm wide.

Oct 15

Worked 8 hours on USGS proposal.
submitted.

SIGNATURE	DATE	WITNESS/TA	DATE
[signature]	10/11/07		

Oct 16.

worked in lab 2 hours w LED. and new mount.

1.) check for 25mm instead of 50mm f.l. lens. ✓ OK.

2.) redo ~~to~~ beam profile plots ✓ OK

3.) perform prelim. bead images

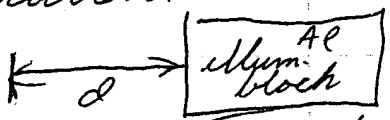
4.) test flow tube for flow! ✓ OK

5.) have Tony mix FITC + shoot images for photometric test!

Oct 17

Worked on time sheets. 1 hour.

took images of LED beam (1ms intg. time) + low current



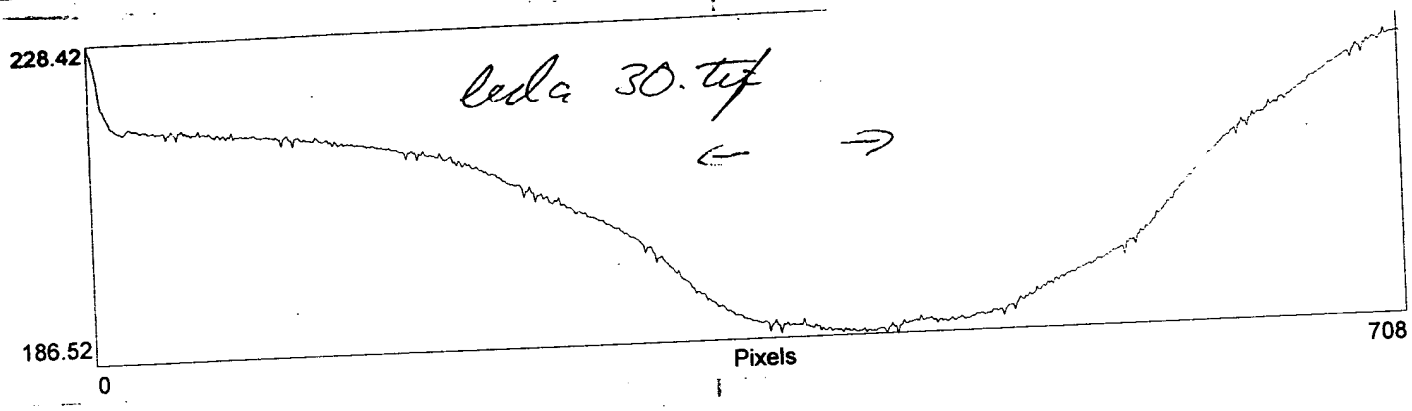
1 ms intg. time

d is distance from Al LED block (w 50mm lens) + CCD surface

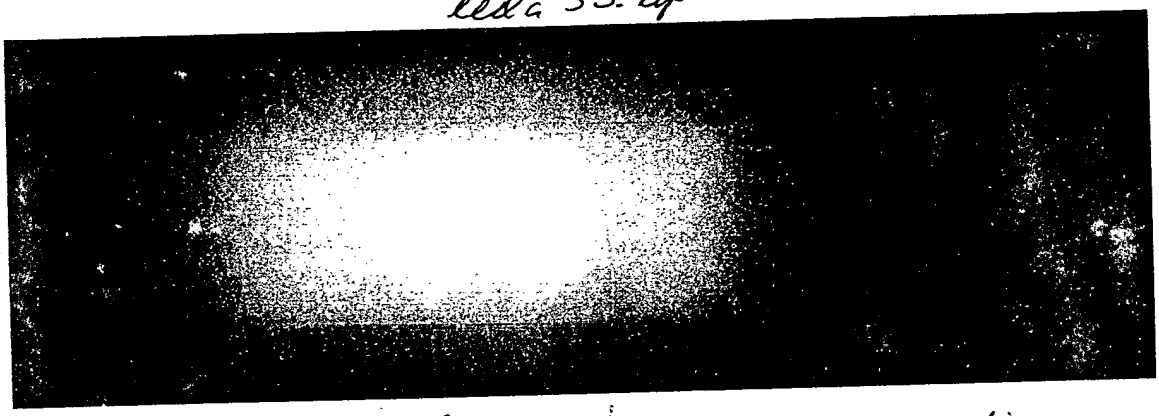
d	image		
30mm	beda 30.tif		
35mm	beda 35.tif	optimum	
40mm	beda 40.tif		
50mm	beda 50.tif		
60mm	beda 60.tif		

focus appears at d = 25mm! Why? Eventually need to test.

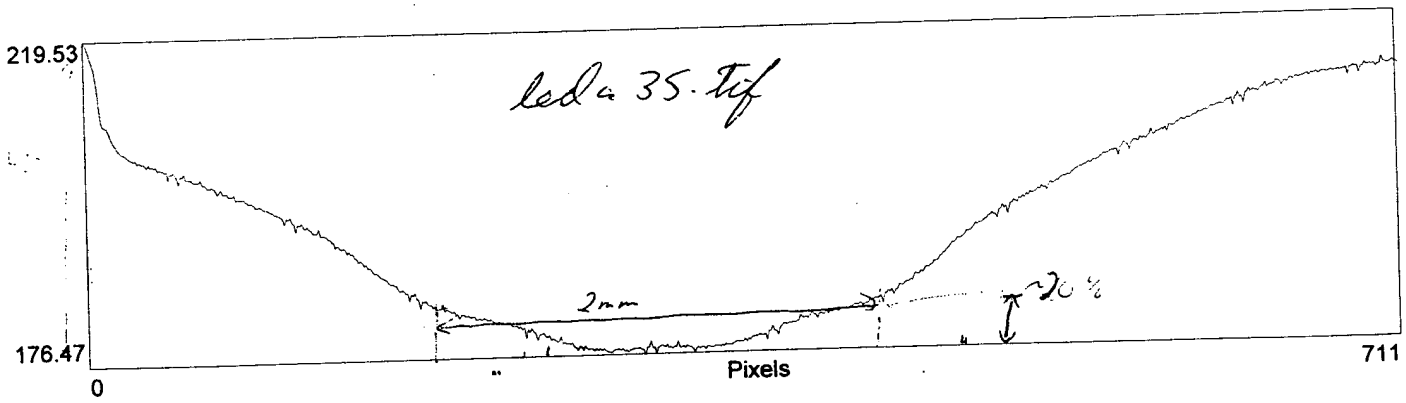
SIGNATURE	DATE	WITNESS/TA	DATE
[signature]	11/11/05		



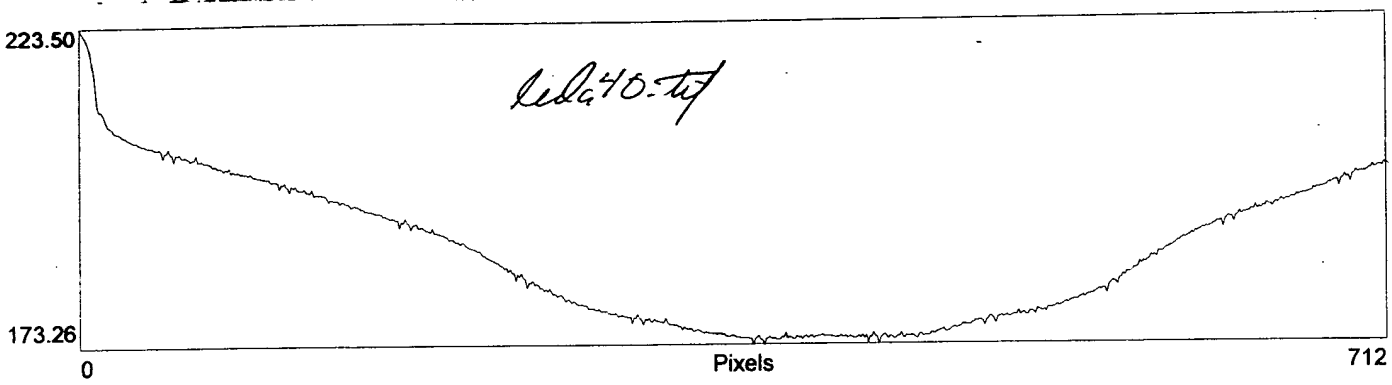
leda 35.tif

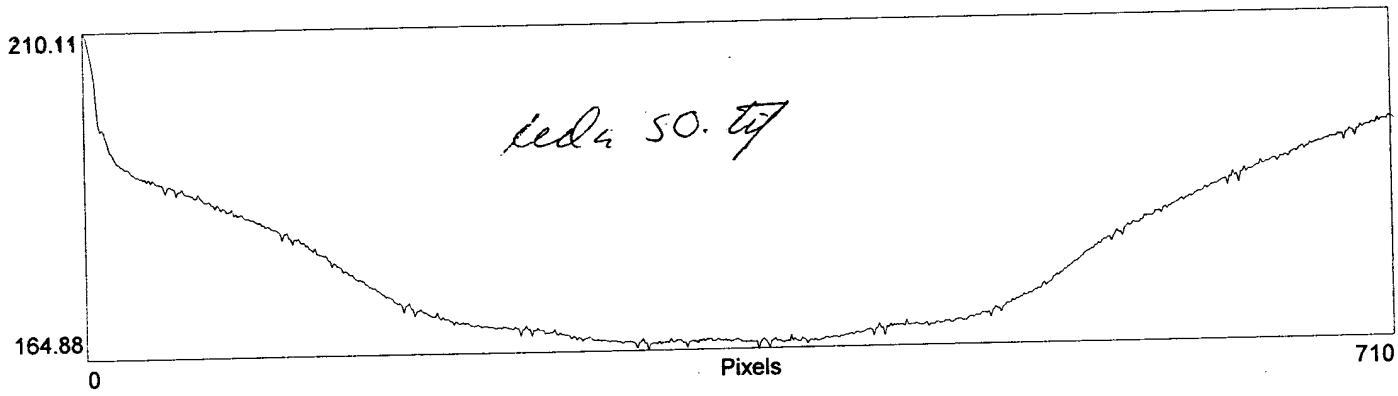


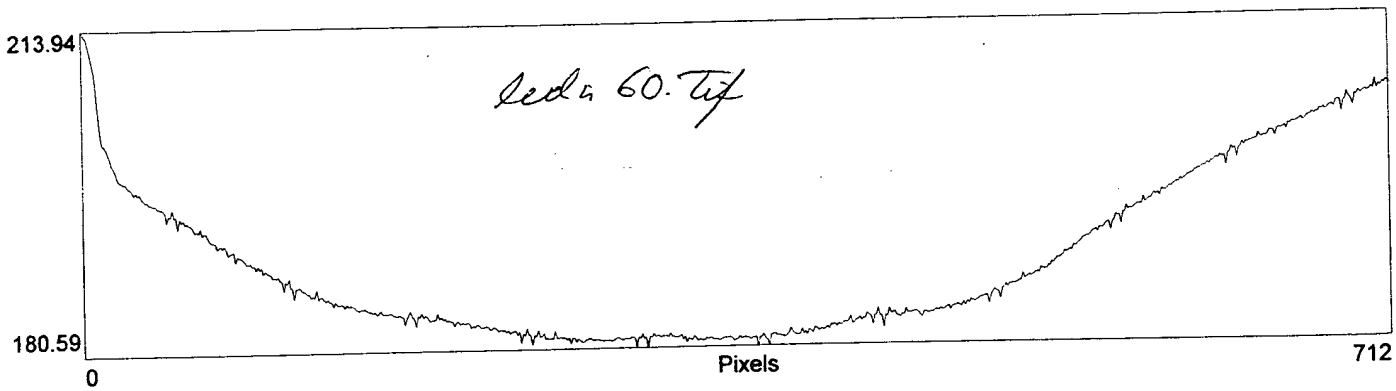
242 x 716 pixels 6mm wide (716 pixels)



SIGNATURE	DATE	WITNESS/TA	DATE
	10/11/02		







SIGNATURE	DATE	WITNESS/TA	DATE
	11/11/01		

>
>Tony,
>
>I worked last night and got the LED to illuminate the flow cell the way
>that I want it and imaged beads. It looks great so far. I want to
test
>this with a fluorescence filter, but I need to order some 1-inch
>filters. My "brilliant" idea with the 45-degree filter didn't work
very
>well, so I'm going to put a 1-inch filter above the imaging optics. I
>did a half-baked test with a 3/4" orange plastic filter. It looks very
>encouraging. I could see beads very well with the power at recommended
>levels (350 milliamps) and exposure times of only 5 milliseconds,
better
>than with our laser.
>
>Anyway, I should have some Edmund filters on Monday and we should have
>the new flow cell materials from the shops then as well. I'll do some
>more tests on Monday and Tuesday, and by the time you return, I should
>know what is going to work, and what is not. I might try to image a
>FITC filled tube as well.
>
>If Gus has the flow cell mounts done tomorrow (please check), you might
>try making a few cells that I can try out next week. Otherwise you
>might try testing stomaching, if there is an overnight available.
>
>We're also going to need Gus to build a better arrangement for holding
>the LED in a stable way that makes adjustment easy and repeatable.
>Using a test tube holder is reaching the end of its useful lifetime.
>
>Anyway,since things are working, I'm going to leave.
>

1

ordered long-pass filters

Nov 2 email from Fruisy re NSF Phase IIB mtg.

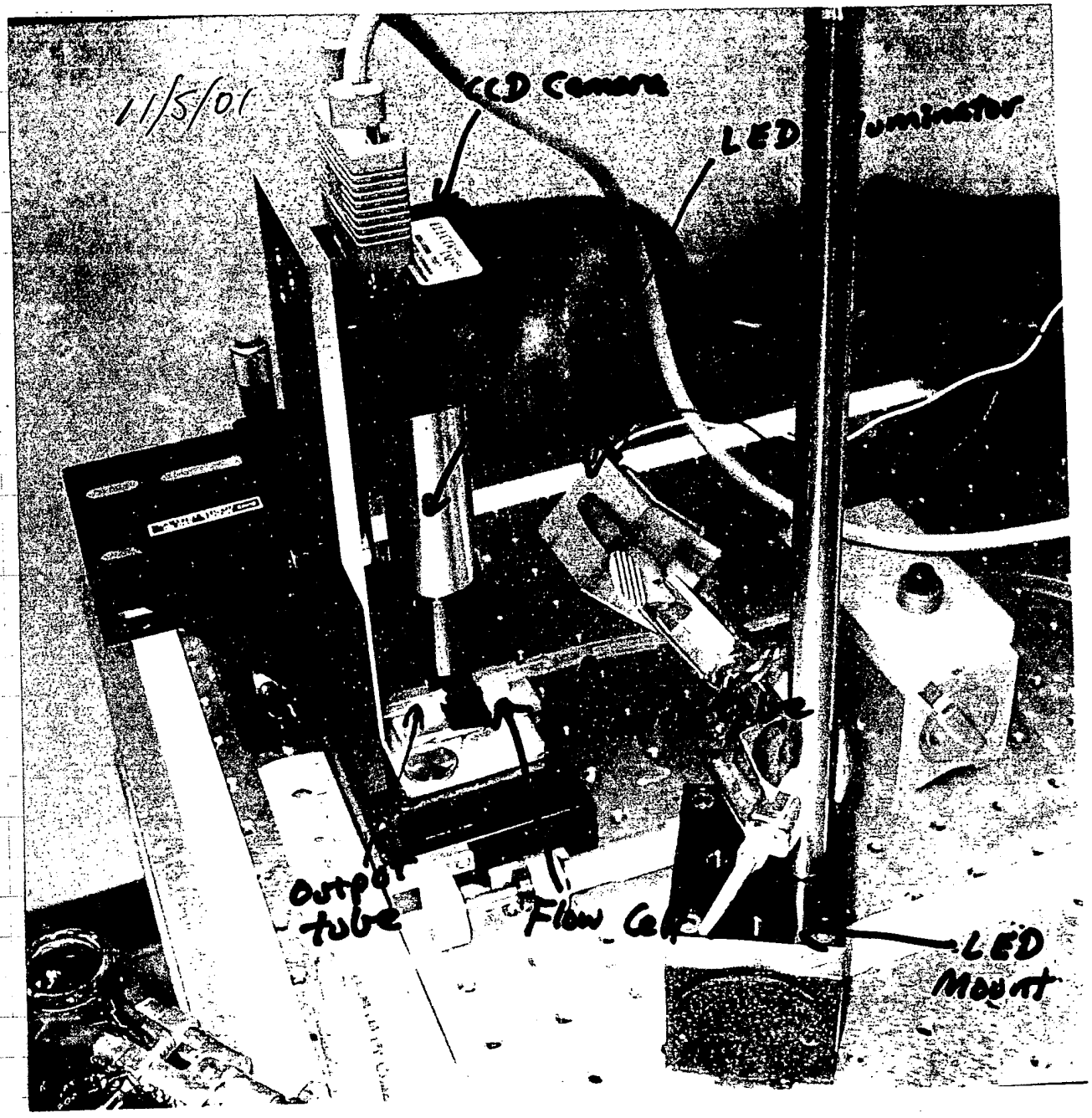
Nov 5

Imaged w longpass filters

see setup below

Received flow cell mounts from machine shop.

SIGNATURE	DATE	WITNESS/TA	DATE
	11/14/01		



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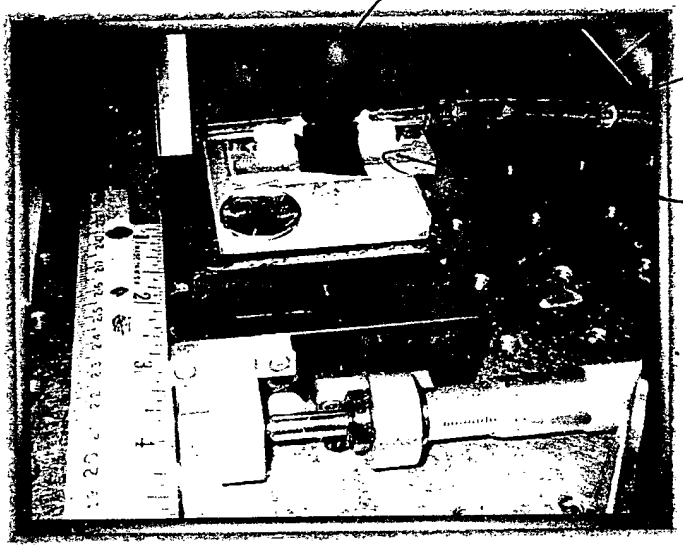
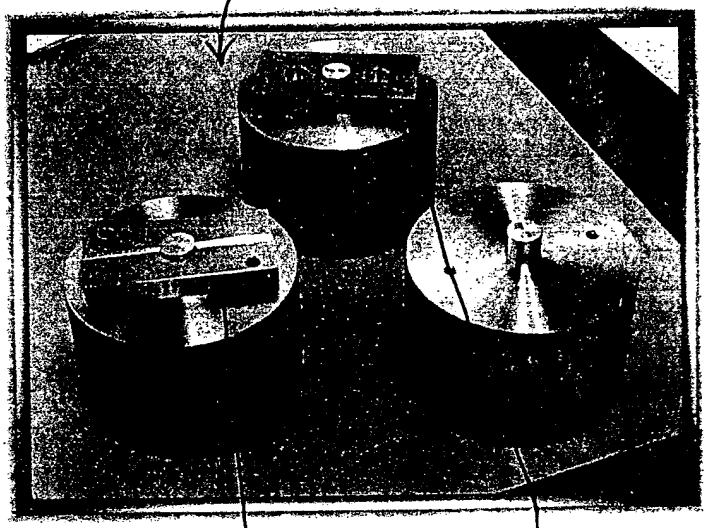
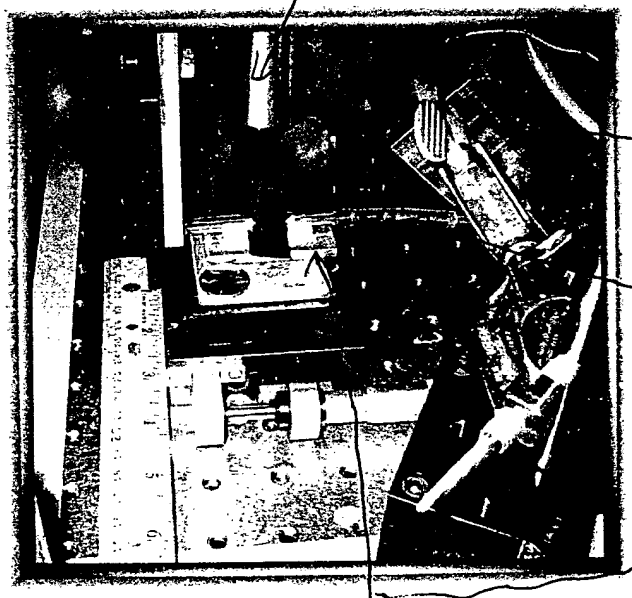
[signature]	11/5/01		

11/05/01







SIGNATURE	DATE	WITNESS/TA	DATE
	11/11/01		

Nov 5 replied to query from Anna Ost regarding experimental controls

Lab mtg.

Nov. 6 email from F.C Edmund Boeing.

Draft. press release from Tech link

ordered Blue Dichroic Filter from Edmund 52-531

Nov. 7 performed Jones experiment gave bracket for LED job to shop

deposit to Bank

Tony built 3 flow cells.

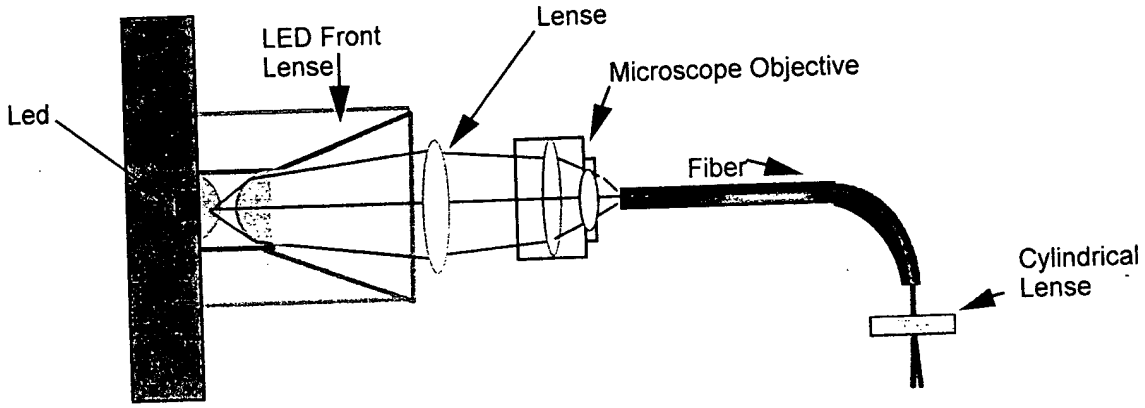
Nov 8 Lab mtg

Experiment w new filter. WORKS.

Discussed budget w Allison

Nov. 9 Dirk sent new optical design (below)

SIGNATURE	DATE	WITNESS/TA	DATE
	11/11/01		



Paul I'll work on puting this together
on saturday or sunday.

SIGNATURE	DATE	WITNESS/TA	DATE
	1/11/01		

[redacted]

Nov. 12

Spent 1 hour w/ Amanda
3 hours time sheets + notebook

Had Gus build mods to LED mount.

Nov. 13

called Cooley + Goodward re representation

called Leo re collaboration

worked in lab w/ Tony looking at beads w/ LED illumination

Nov. 14

made calls searching for IP attorney

spent 2 hours in lab w/ Tony looking at beads

called Michael Johnson

Nov. 15

called Leo
ordered letter
Lab mtg
talked to Gus re spectrometer
spent 2 hours in lab w/ Tony imaging beads. TDI works.

Nov. 16

called Colbey + George re getting prologon.

SIGNATURE	DATE	WITNESS/TA	DATE
~~2/16/04~~	2/17/04		

talked to Biozan about meeting - worked in lab w/ Tony

Nov. 17 shot pictures in lab

Paul E. Johnson

From: Michael G. Johnson [mjohnson@uark.edu]
Sent: Wednesday, November 14, 2001 11:38 AM
To: Paul E. Johnson
Cc: Mjohnson@Uark. Edu; Rama@Uark. Edu
Subject: RE:

Dear Dr. Paul Johnson:
Do you have a street address for package delivery ? If so please send by return email.
The two more recent papers outlining the limitations of our 7G1 monoclonal antibody for L.m. are covered in two pubs by my colleague, Dr. Rama Nannapaneni as follows:
Nannapaneni, R. et al 1998. J. Food Protection 61: 1195-1198 and Nannapaneni et al 1998. Applied Enviriomental Microbiology 64:3070-3074.
If you would send me a request for some of the 7G1 and mention the reason you want to use it, ala the NSF-SBRI grant proposal and formal title of same, I can then send you a letter of cooperation and some of this antibody for you to test.
Good luck in your application. Sincerely, Mike Johnson, Prof., Food Science Dept., U of Arkansas
-----Original Message-----
From: Paul E. Johnson [mailto:PJohnson@uwyo.edu]
Sent: Wednesday, November 14, 2001 10:34 AM
To: mjohnson@uark.edu
Subject:

Dr. Johnson,

Thank you for taking the time to talk to me this morning. We are looking forward to working with your antibody. My address is:

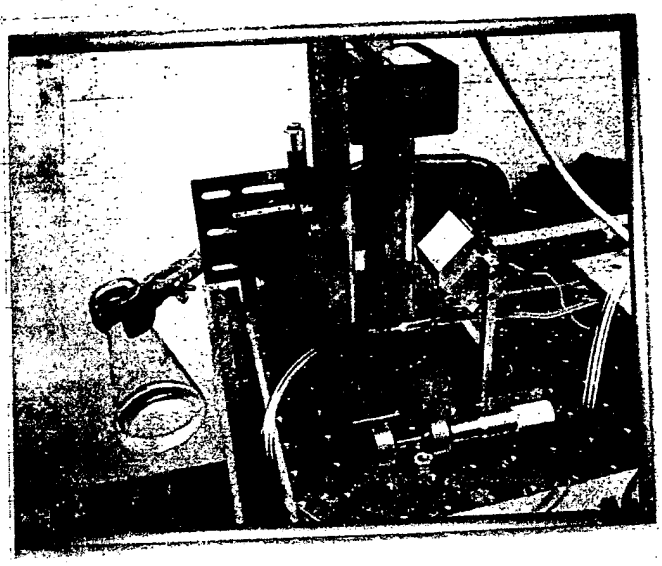
Dept. of Physics and Astronomy
University of Wyoming
Laramie, WY 82070

My best,

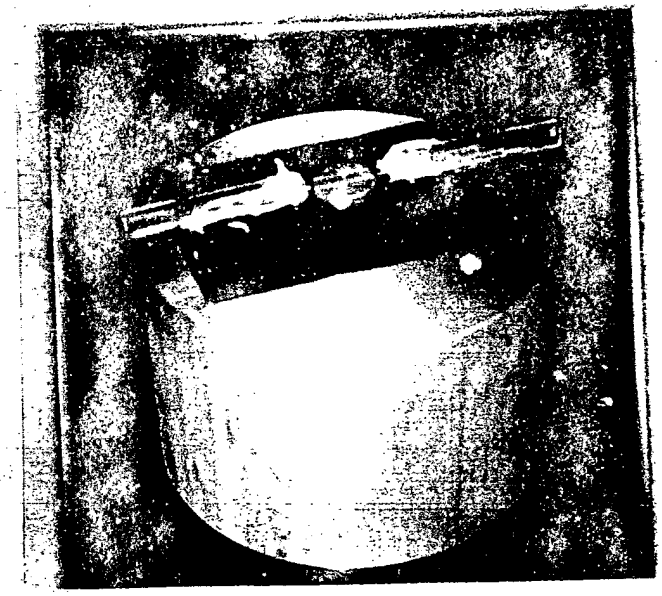
Paul

SIGNATURE	DATE	WITNESS/TA	DATE
	2/18/02		

Nov 17 2001
images

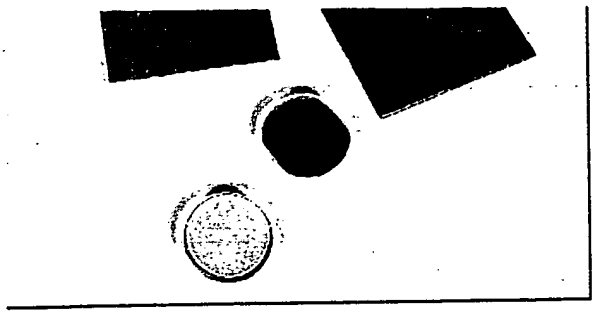


↑
new LED illuminator illuminating flow cell, imaged from above



← flow cell constructed on jig w RTV (silicone element).

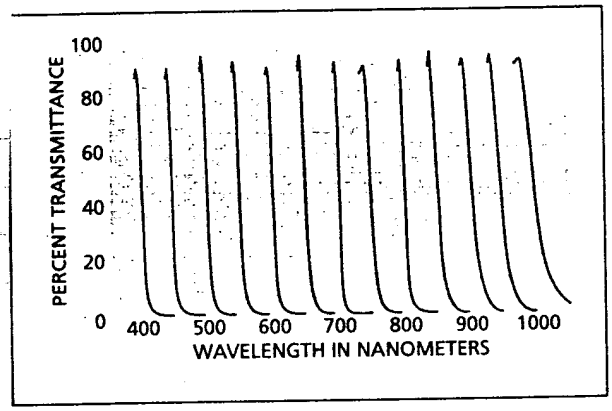
SIGNATURE	DATE	WITNESS/TA	DATE
[signature]	2/18/02		



Short-Pass Filters

Melles Griot short-pass filters, also called edge filters, have harp cutoff transitions from reflection to transmission.

- The filters have a steep cutoff edge.
- They are useful for attenuating specific wavelengths.
- They are available in 50-nm increments from 400 nm to 1000 nm.



Typical transmittance curve

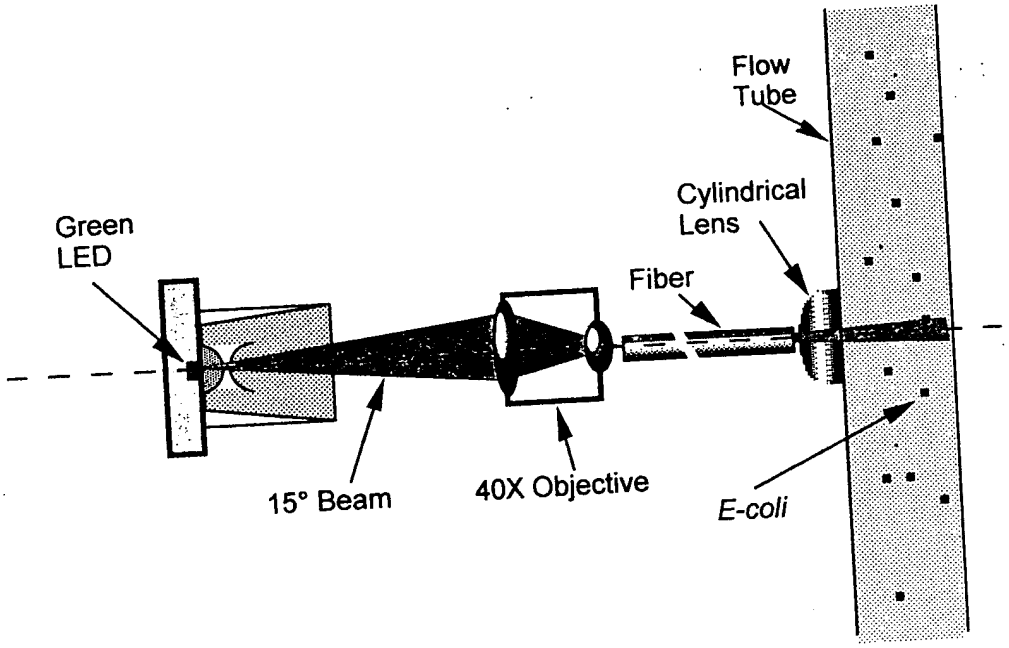
SPECIFICATIONS: SHORT-PASS FILTERS

Dimensions:
Diameter: 25.0 +0, −0.15 mm
Square: 50.0 mm × 50.0 mm (±0.25 mm)
Thickness: 1.5 ± 0.5 mm
Clear Aperture:
21 mm for round, 90% of edge dimensions for square
Substrate Material: Fused silica
Wavefront Distortion:
1λ peak to valley per 25-mm area at 632.8 nm over clear aperture
Parallelism: 15 arc seconds maximum
Surface Quality: 80-50 scratch and dig
Operating Temperature: □ 20°C to +200°C
Cutoff Tolerance: □ 10 nm
Angle of Incidence: 0°
Transmission: 85% average over specified wavelength range
Rejection:
99% average, except for 400–450 nm range which is 98% average
Coating:
Antireflection coating R ≤0.75% over transmission range

Short-Pass Filters

50% Cut-off Wavelength (nm)	Typical Rejection Range (nm)	Typical Transmission Range (nm)	PRODUCT NUMBER 25.0 mm Round	PRODUCT NUMBER 50 mm□ 50 mm Square
400	420–470	285–390	03 SWP 402	03 SWP 602
450	470–530	310–440	03 SWP 404	03 SWP 604
500	525–610	340–485	03 SWP 406	03 SWP 606
550	570–680	375–540	03 SWP 408	03 SWP 608
600	620–790	400–585	03 SWP 410	03 SWP 610
650	675–850	400–640	03 SWP 412	03 SWP 612
			03 SWP 414	03 SWP 614

SIGNATURE	DATE	WITNESS/TA	DATE
	2/18/02		



Paul E. Johnson

From: Paul E. Johnson
Sent: Tuesday, November 20, 2001 11:01 AM
To: 'Richard W. Shorthill'
Subject: RE: Brightness

Dick,

Some comments on your drawing:

We have to illuminate the bugs from above (light hitting the broad side of the rectangular tubing), and image them from above. Any technique that places the cylindrical lens on top of the broad side of the tube won't be viable. The illumination has to come in at an angle. Any technique that places the lens on the narrow side of the tube won't work either (the beef extract is opaque over a thickness of 2mm). Secondly, why use a cylindrical lens? The field of view of the CCD has a 6/4 aspect ratio so a square or circular illumination patch is completely satisfactory. Finally, the way this is drawn, you have a 15-degree beam, when in fact we have something like a 110-degree beam. Can you find a microscope objective with a short enough focal length (fast enough f/#) that you actually intercept most of the light emitted by the LED?

Finally the illuminated patch on the optical tube should ideally be about 2mm by 2mm square (perhaps slightly larger) and collimated (or at least not highly divergent over the 100 micron depth of the flow tube).

Paul

[redacted]

Oct 21 commicated w Anne Ost

Oct. 23

w/ Whipple Grid

From Nick's measurements w scanning objective

FOV = 1.15mm x 1.15mm ?

10x obj. ⇒ 0.46mm x 0.46mm

20x .23mm x .23mm

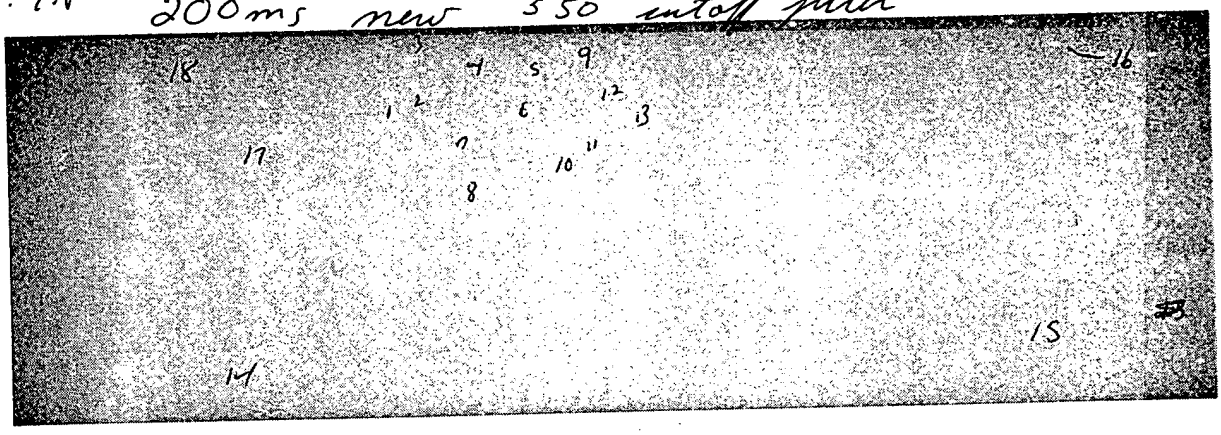
normally use ⇒ 40x .115mm x .115mm

100x .047mm x .047mm

40x Whipple Grid area 0.013225 mm^2

With Tony took Carmine PeckAlign Bead (Probes) image. ✓✓
used Edmund 590nm longpass filter (1")

bead 1.tif 200ms new 550 cutoff filter



some beads stuck together
nearly uniform! surprisingly so!
prelim results ±.08 STDEV
(in excel)

14 .59
15 .99 12 = .4

Sent term sheet to Osman

SIGNATURE	DATE	WITNESS/TA	DATE
[signature]	2/18/02		

summary of bead1.tif photometry (Nov.?) 12/23/01

# particles stuck together	1	2	3	4
	0.4	0.85	1.11	1.96
	0.42	0.79		1.83
	0.57	0.71		
	0.59	0.77		1.89 avg
	0.4	0.84		
		0.92		
	.47 avg ± .05	0.99		
	{ 0.096 STDEV (in excel)	0.78		
		0.89		
		0.92		
		.84 avg ± .03		
		{ 0.085 STDEV (in excel)		

Bead

1. .85
2. .40
3. .79
4. .42
5. .40
6. .57
7. .71
8. .77
9. .84
10. 1.78 ÷ 2 = .89 } double beads
11. 1.84 ÷ 2 = .92 } double beads
12. 1.96
13. .92
14. .55
15. .99
16. .78
17. 1.11
18. 1.83

Photometry (- background) using Scion

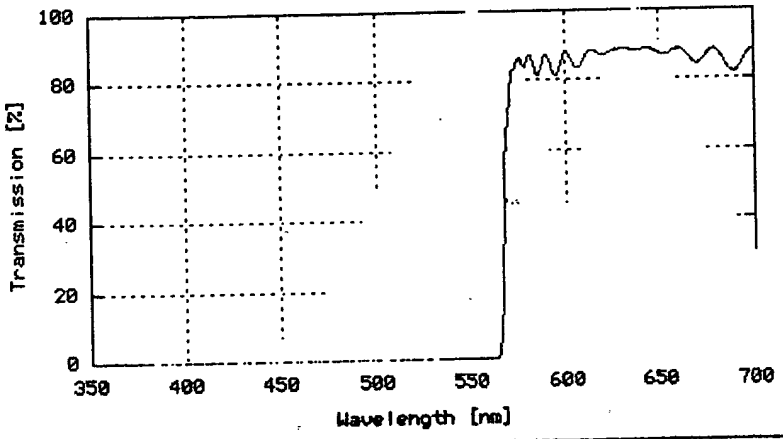
SIGNATURE	DATE	WITNESS/TA	DATE
	2/18/02		

OMEGA OPTICAL incorporated

Do not have this, but we should order something similar but about 10nm shifted to red, for emission filter.

Curv-o-matic

Component **XF3085** (565ALP)
Fluorophore **Nile Red** *
Compatible: NO



SIGNATURE	DATE	WITNESS/TA	DATE
	2/18/02		

[redacted]

11/[redacted]/01
11/26/01

Set up mtg. with Osman + Humbeiss.
Forwarded necessary info.

2-hours Worked w Tony in lab starting TDI.

Set up travel to France.

Met w Gus regarding spectrometer.
optical design site www.astron.de

11/27/01

Worked w Tony in lab. He is starting to ~~count ramonine beads~~ do TDI.
Drafted BAA ~~Ltr~~ draft for Leo.
sent.

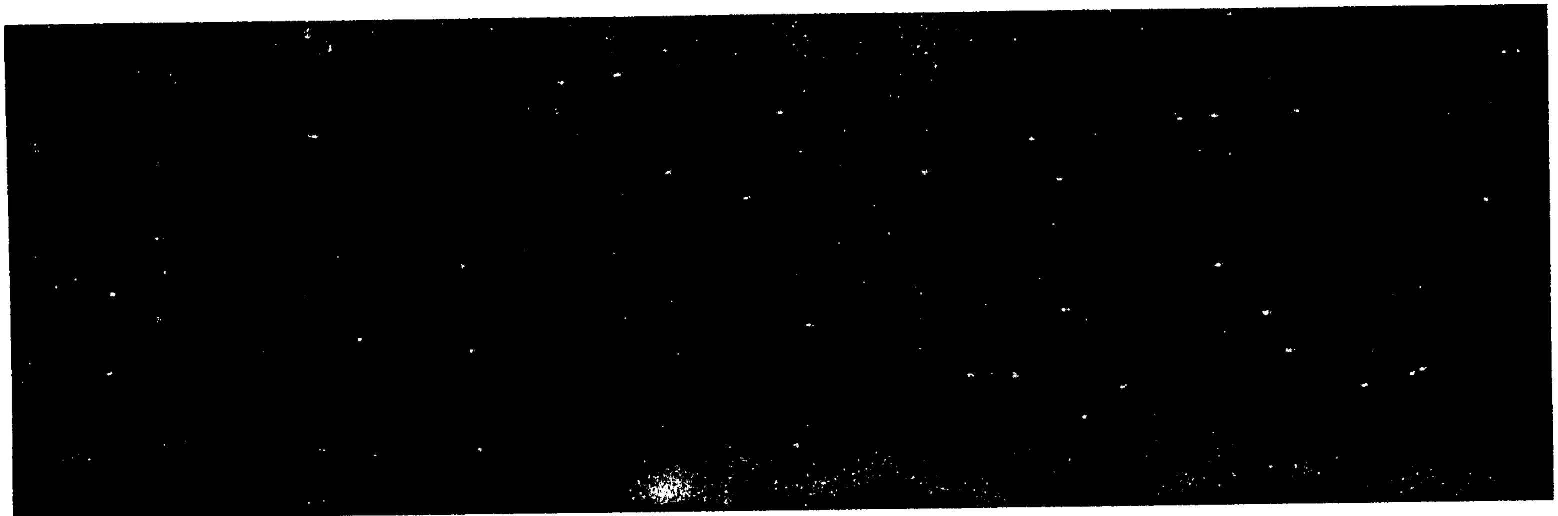
11/28/01

Worked w Tony in lab setting up for TDI. need depth of focus measurement.
Telecon w Dorsey Whitney

11/29/01

Lab meeting.
met w Ryan + Tony in lab.

SIGNATURE	DATE	WITNESS/TA	DATE
[signature]	2/18/02		



Carmine beads   in focus   1129FD3.tif

1129FD5.TIF   200 um down

SIGNATURE | DATE 2/18/02 | WITNESS/TA | DATE

[redacted]

11/30/01

email exchange w Anna regarding contract

Worked w Tony in lab.

Tony ran various experiments:

Paul E. Johnson

From: Tony Deromedi [ajd65@hotmail.com]
Sent: Friday, November 30, 2001 5:43 PM
To: Paul E. Johnson
Subject: beads

Paul - I used the hemacytometer to find the concentration and I got 1.32 X
10^7 and molecular probes documents showed 1.7 X10^7, so we are as close as
you can get. The hemacytometer works way better then the whipple grid, so I
guess I'll use it in the future.

Anyway, I ran the 6.6 X 10^4 carmine beads through the flow tube varying

intensities, flow rate, and exposure time. The results are on a CD that I
will leave for you in the physics office. I will also leave the image
numbers to which they correspond to with the CD. It seems that the pump

rate works the best on 15, at 7.5 no beads were seem even with 350000 and 6
amps. Take a look and see what you think. Give me a call tomorrow at home
if you want. 745-4603.

Have a nice evening.

Tony

Worked intensively on patent ~~[illegible]~~ searches.

11/31 email exchange w Jennifer

SIGNATURE	DATE	WITNESS/TA	DATE
[signature]	2/18/04		

Paul E. Johnson

12/2/01

From: Tony Deromedi [ajd65@hotmail.com]
Sent: Sunday, December 02, 2001 11:05 AM
To: Paul E. Johnson
Subject: beads

Hey Paul,

I came in today to try some imaging on the beads. I took a few images with
the EDC camera varying the focal distance 70 um up and down. They are saved
under d0201(in focus), d0202 (70 up), d0203(70 down). They looked pretty
good so like we thought the focusing isn't really our issue. I think we

need to figure out if we are getting accurate counts now. I don't know the
most efficient way to do this though.

I'm leaving my lab notebook in the lab with the CD that I made on friday.
The images are listed in my lab notebook. I didn't have a key to the physics department so I couldn't leave the results for you on friday. Sorry
about that.

I'll be in tomorrow at 2 so we can talk about it then. Have a nice day.

Tony

12/3/01
received Ryan's photomerly on
in + out of focus images
presentation needs to be reworked
worked in lab w Tony + Ryan
* met w David Sanguett re new IP

SIGNATURE	DATE	WITNESS/TA	DATE
	2/18/02		

[redacted]

12/4 and 12/5 ⇒ worked on IP ☆

Paul E. Johnson

From: Paul E. Johnson
Sent: Wednesday, December 05, 2001 1:54 PM
To: Amanda S. Votaw; 'a_votaw@yahoo.com'

Amanda,

The measurements are starting to go extremely well!

1. We started by taking a stock solution of beads and measuring the concentration with the hemocytometer (Tony doesn't like the Whipple Grid). We get 7.1 x 10^4 beads/cc.

2. We took a series of still images of beads in the flow cell (having directly measured the field of view and cell depth). We converted these to a concentration. We get 7.2 x 10^4 beads/cc!

3. Now we flow the same syringe of beads into the flow cell and do TDI. We see about 90% of the beads! Now we're tuning the flow speed and integration time to see if we can improve from 90%.

We've already done multiple runs and we're getting the same answers. I'm getting Ryan to do bead photometry to see how uniformly bright they are in the images. Tony is starting to label K-12 so that we can repeat these measurements with bacteria. It feels like we've crossed the major hurdles on this project.

Paul

12/6

Tony signed IP ☆

Lab mtg.

Abstract to Alan Abend's meeting.

Ryan signed IP ☆

Amanda reviewing IP

SIGNATURE	DATE	WITNESS/TA	DATE
[signature]	2/18/02		

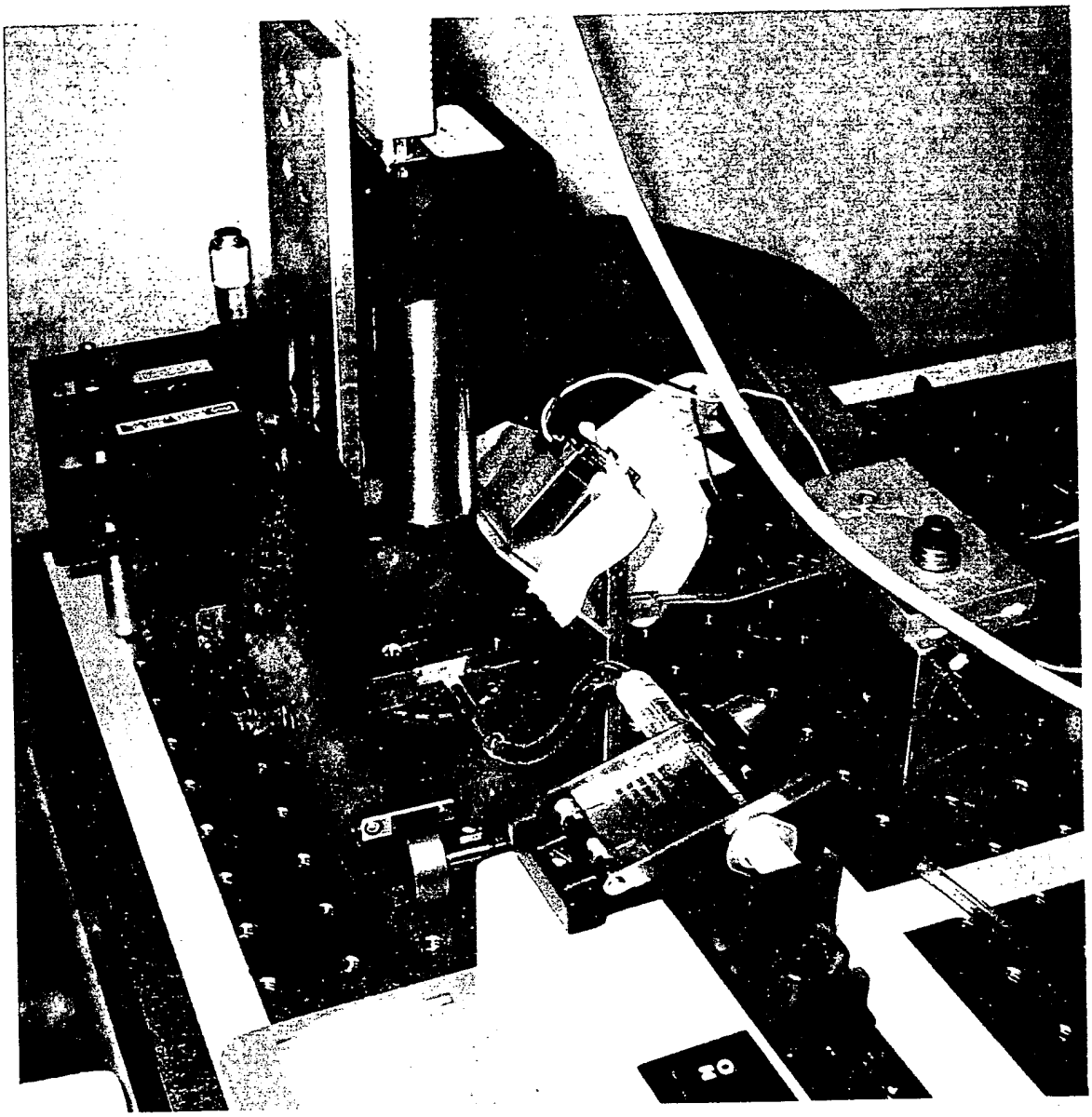


Figure 3. The aluminum flow block shown in figure 2 is shown here mounted on a rotary optical mount. A glass covers slip constrains the liquid flow out of the exit hole in the aluminum block. A CCD camera (black, upper portion of figure) is mounted vertically and connected to fore-optics with an aluminum mounting tube. A fluorescence filter is taped over the fore-optics with electrical tape. Fluorescent beads are pumped into the flow block with a syringe pump. The mouth of the exit hole is illuminated with an LED illuminator (the cube-shaped aluminum block with blue tape on the side).

12/31 worked on France travel
D.E. Geomet revision
met w Allison

Jan 2 Worked on Geomet MOU
w Richard
Sent draft below

Draft Minutes of Meeting between SoftRay and BD, December 21, 2001

Present: Noel Warner, Bob Hoffman, Dieter Recktenwald, Jacleen Schmidt, Krissy Manion & Paul Johnson

Recommendations:

1. Marketing of the SoftRay Cytometer must be examined in January for the food processing market in order to enable focused planning for SoftRay R&D, a decision from BD on NSF Phase IIB funding, and to specify more precisely the goals for development of the device, specifically over the next 6 months. Issues to be examined in order to make the device marketable at an acceptable level in the food processing market, including areas outside of ground beef, include:
    a. required minimum flow rate of the device,
    b. required sensitivity, specificity, efficiency of detection, and false positive rate, and
    c. number of simultaneous colors required (probably R-PE and an R-PE conjugate such as CY5)
2. Specifically, to focus R&D of the device, SoftRay should:
    a. during the next month examine image background issues, e.g. by looking at Quantibrite beads (supplied by RH) in an R-PE background,
    b. illuminate beef extract with LED and image beads, then bacteria to further test background issue,
    c. obtain R-PE conjugate from Ken Davies,
    d. test axial flow vs. TDI to eliminate one
    e. design and machine two-color device using two image/ two color configuration,
    f. check into filters (AF) for LED, and
    g. consider increasing the brightness of LED or use multiple LEDs if necessary.

Dear Dr. Paul Johnson:
Do you have a street address for package delivery ? If so please send by return email.
The two more recent papers outlining the limitations of our 7G1 monoclonal antibody for L.m. are covered in two pubs by my colleague, Dr. Rama Nannapaneni as follows:
Nannapaneni, R. et al 1998. J. Food Protection 61: 1195-1198 and Nannapaneni et al 1998. Applied Enviriomental Microbiology 64:3070-3074.
If you would send me a request for some of the 7G1 and mention the reason you want to use it, ala the NSF-SBRI grant proposal and formal title of same, I can then send you a letter of cooperation and some of this antibody for you to test.
Good luck in your application. Sincerely, Mike Johnson, Prof., Food Science Dept., U of Arkansas

From: Ost, Anna [mailto:Anna.Ost@AirLiquide.com]
Sent: Thursday, January 17, 2002 6:29 AM
To: Paul E. Johnson
Subject: RE: 5.30 Monday 14 January

Dear Paul,
It was nice to see you again! I hope your trip was pleasant both personally and professionally! I am sorry that I could not speak correctly but I think the meeting went well anyhow.

I would like to give you some indications that would shorten the negotiation process so that we can really start to work!

1. Generally, all suggestions you evocated seemed fair to us.
2. The down-payment that you mentionned is ok for us if it could include the first 25 units sold/put in use.

We should also start to discuss the material, for instance could one easily change filters/LEDs for different dyes? What are the dyes that you have already tested?

If we sign the contract say in one month (mid-february), when could you install the equipment do you think?

My best to the whole SoftRay-team

Anna

SIGNATURE	DATE	WITNESS/TA	DATE
	2/18/02		

EXHIBIT J

Tony Deromedi

Tony Deromed.		

1/30/01 Intesity Experiment / Pump Rate / Exposure time

-WILL PERFORM TBI on 6.6 x10⁴ commie beads
varying LED AMPS (.3 - .6), Pump Rate (15 - 7.5 psi)
Exposure time (700,000 - 350,000)

				#filename 1130 Image # 15
1) 20 IMAGES →	.3 AMPS	700,000	15 psi	1-20
2) 20 IMAGES →	.6 AMPS	700,000	15 PSI	21-50 ☆
3) 20 IMAGES →	.3 AMPS	700,000	7.5 PSI	67-90
4) 20 IMAGES →	.6 AMPS	700,000	7.5 PSI	#filename 1n30 1-20
				CN30
5) 20 IMAGES →	.3 Amps	350000	15 PSI	1-25
6) 20 IMAGES →	.6 Amps	350,000	15 PSI	25-60 ☆
7) 20 IMAGES →	.3 AMPS	350000	7.5 PSI	75-100
8) 20 IMAGES →	.6 AMPS	350000	7.5 PSI	100-150

→ Beads were stirred for 30 minutes then sonicated for 4 minutes then vortexed for 1 min

			#filename CN30	
20 IMAGES	.3 AMPS	700000	25 PSI	1-30
20 IMAGES	.6 AMPS	700000	25 PSI	30-60
			CN30	
	.3 AMPS	700000	25 PSI	1-30
	.6 AMPS	700000	25 PSI	30-60

5) 7.5 PSI → NO IMAGES -Maybe try stretching them or something

SIGNATURE	DATE	WITNESS'TA	DATE

JDW Journal

12/02/01: Stored 6.6 x 10⁴ Carmine beads for 30 minutes, sonicated for 4 min, Vortexed 1 min.

Ran small amount through flow cell.

Saved Images to EDC software:

D0201 → Infocus
D0202 → 70um up
D0203 → 70um down

→ focus really isn't the issue.

12/03/01: Measure Beads with hemacytometer →

→ 10 still frames d3s (1-10) (.6 Amps)

→ 125 image (.6 Amps)(700,000)(15 PSI) d03t (1-125)

→ 10 still frames d3s (11-20)(.6 Amps)

→ 125 imags (.6 Amps)(700,000)(15 PSI)

1	11	6	14		
2	11	7	7		
3	9	8	12	89/10 = 8.9 x10⁴	8.9 x10⁴
4	2	9	7		
5	9	10	7		

SIGNATURE	DATE	WITNESS/TA	DATE

Tony Desmell

12/05/01: VAVG Integration time from 2.1 million X .20 = 420000

1.86 million — 2.52 million
1.44 million — 2.94 million
1.02 million — 3.36 million

@ .6 AMPS & 5 PSI.

2.1	d05a	1-40 - Nothing
2.52	d05B	1-20 - Nothing
2.94	d05C	1-20 - Nothing
3.36	d05D	1-20 - Nothing
1.86	d05E	1-10 - Nothing
1.44	d05F	1-10 - Nothing
1.02	d05G	1-10 - Nothing
4.00	d05H	1-10 nothing
1.15	d05I	1-10 nothing
~~1.15~~	~~d05~~	
1.20	d05J	1-10 nothing

12/06/01: Lab meeting

- Try to put FITC Ab on K12.
- Followed protocol for Ab labelled
- Can't see anything

SIGNATURE	DATE	WITNESS/TA	DATE

Tony Dennis

12/19/11 Setup New Flow cell.

Saved Still Images under

.6 AMPS 3 PSI ⟶

1214 NC → No coverslip
1214 NC 2 →
1214 CS 1 →
1214 CS 2 →

Took 100 Images under IDL
@ 3 PSI & .6 AMPS

Filename

1214 C 1 - 100 — coverslip → Look good;
1214 N 1 - 100 → No coverslip →

→ Aimed well with Black Ant
took ten more images.
May be less background scattered light.

SIGNATURE	DATE	WITNESS/TA	DATE

Tony [illegible]

1/24/02: General Lab cleanup + spike in [illegible] R phycoerythrin
Stirred 10^6 [illegible] bead for 30 min
Sonicated for 5 min
Vortexed 1 min

- WILL TRY AGAIN TO CHANGE GAIN + BIAS ON IDL software to see if images are better. using FF

20 images of each @ 300 Exposure time + 15 PSI

Gain 50 / Bias 100 — 0124a
Gain 50 / Bias 125 — 0124b
G 50 / B 150 — 0124c
(G) 50 / (B) 175 — 0124d
Gain 50 / Bias 200 — 0124e

} All seem ~~[illegible]~~ SIMILAR

Gain ~~200~~ 150 / Bias 200 — 0124f
Gain 150 / Bias 175 — 0124g
Gain 150 / Bias 150 — 0124h
Gain 150 / Bias 125 — 0124I
Gain 150 / Bias 100 — 0124J

} All the same

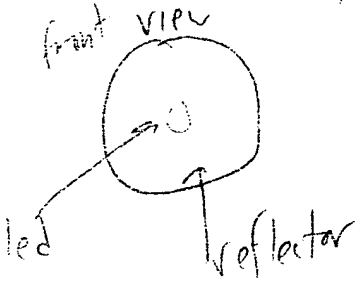
SIGNATURE	DATE	WITNESS/TA	DATE

EXHIBIT K

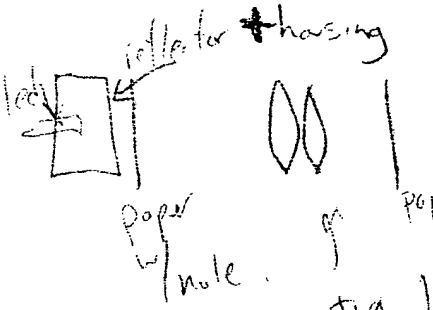
(Nick Deskevich)

EXP. NUMBER	EXPERIMENT/SUBJECT	DATE	33
		7 26 01	
NAME Nick Deskevich	LOCKER/DESK NO.	COURSE & SECTION NO.	

LEDs finally came
they project much like a flashlight
try to ~~foc~~ ~~focu~~ focus down to a point.



put a piece of black paper over the led. Paper has a hole in it. This reduces amount of light spreading out.



used two lenses to focus beam. Everything held together w/ wooden sticks, black paper and tape.

two lenses (see p 31)

This setup focuses down to a spot. Only works at a fixed distance unlike a laser. This can be changed by moving the lenses. A laser line generator in front produces a ~~focused~~ focused line about 3mm long.

Valid 1/8/02

SIGNATURE	DATE	WITNESS/TA	DATE

EXP. NUMBER	EXPERIMENT/SUBJECT	DATE	34
		7 31 01	
NAME NICK DESKEVICH	LOCKER/DESK NO.	COURSE & SECTION NO.	

The green LED focused down to a line seems like it will light up the right size and shape part of the flat cell. My line is slightly uneven but with more careful construction i.e. not sticks, paper, and tape, a better line could be produced. I will hold off making something better until I can get a better filter. The filters I have available work for the green laser (532?) but they pass through a bunch of LED light. I also don't know if the LED will be bright enough to be effective. A lot of light is lost in my blocking and focusing set up. I tried 7, 9, and 24 LED arrays but when focusing I get individual dots (7, 9 or 24) when I block them off, I only get the light of one LED from the array. A single super bright LED like the one from Lumileds seems to be the best way to go.

Valid 3-8-07

SIGNATURE	DATE	WITNESS/TA	DATE

EXHIBIT L

September 21, 2000

LED types:

(532 nm) Green Small → NSPG300A ①
Large → NSPG500S ②

(450-495 nm) Bluish-Green Large → NSPE590S ③

	Optical Power Output (mW)	DC Forward Voltage (V) Typ	DC Forward Voltage (V) Max	DC Reverse Current (μA)	Power Dissipation (mW)	Luminous Intens. (cd)	Dir *
①	4	3.5	4.0	50.0	120	6.80	15°
②	4	3.5	4.0	50.0	120	18.00	15°
③	4-6	3.5-3.6	4.0	50.0	120	?	

* Directivity 2 theta ; 1/2

For ALL:

DC Forward Current = 30 mA ←
Pulse Forward Current = 100 mA
DC Reverse Voltage = 5V
Operating Temp = -30 ~ +85
Storage Temp = -40 ~ +100

* Run at 20 mA, 25 mA, 30 mA
↑
(For short time) → MAX

✓KHB
11-06-00

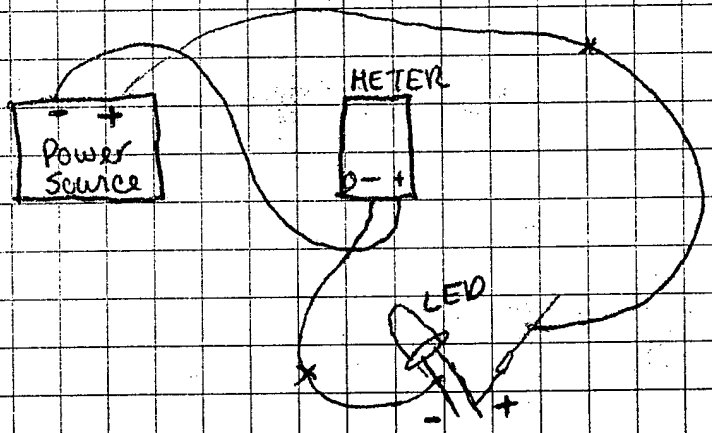
September 28, 2000

- Biohazard Training
- Test LED's (Find 25 mA)
- New flatpack LED's in → NSPGF505

October 5, 2000

Large Green (NSPG5005) (w/o brass casing)

LED #1 → Visible Light @ .03 mA



Current (mA)	Power (mW) → Measured fr. Laser Check (right next to LED)
0.03	—
5.03	0.79
10.02	1.35
15.01	1.84
20.06	2.18
25.04	2.77
26.57	2.82

VKHB
11-06-00

Large Bluish-Green LED (NSPE590S) (w/o Brass Casing)

LED #2 (w/o resistor)

Current (mA)	Power (mW)
5.04 ± 0.05	0.33 ± 0.02
9.97	0.61
15.08	0.87
20.16	1.13
24.99	1.34
30.10	1.52

Small Green (NSPG300A) (w/o Brass casing)

LED #3 (w/o resistor)

Current (mA)	Power (mW)
5.02	0.41
10.02	0.67
15.02	0.87
20.13	1.06
25.02	1.22
30.05	1.34

JKB
11-Dec-00

October 11, 2000

LED #4,1 → Large Green (NSPG 500S)
LED # 5,2 → Large Blush-Green (NSPE590S)
LED # 6,3 → Small Green (NSPG300A)

LED #2 (w/ resistor) (w/o brass casing)

Current (mA)	Power (mW)
4.65	0.32
9.67	0.64
15.00	0.82
19.46	1.06
25.60	1.34
30.~~78~~	1.~~49~~9

LED # 5 (w/resistor) (w/o brass casing)

Current (mA)	Power (mW)
4.89	0.35
10.54	0.62
14.03	0.98
20.18	1.22
24.63	1.50
29.92	1.74

LED # 3 (w/resistor w/o casing)

Current (mA)	Power (mW)
5.50	0.46
9.58	0.68
14.19	0.88
19.35	0.99
24.75	1.31
30.03	1.45

LED #6 (w/resistor w/o casing)

Current (mA)	Power (mW)
5.74	0.43
9.68	0.58
14.56	0.80
19.80	1.01
24.61	0.98
29.45	1.29

✓KHB
11-06-00

LED #4 (w/resistor) (w/o casing)

All Power readings taken using Laser Check

Current (mA)	Power (mW)
4.86	0.89
10.20	1.86
15.03	2.44
20.10	2.82
24.71	3.37
≈ 29.00	3.74

LED #7 Large Green (NSPG500S)
(w/resistor) (w/o casing)

Current (mA)	Power (mW)
~~[illegible]~~	~~[illegible]~~
5.05	0.98
10.21	1.78
14.57	2.22
20.03	2.82
24.40	3.15
≈ 29.97	3.85

LED #8 Large Green (NSPG500S)
(w/resistor) (w/o casing)

Current (mA)	Power (mW)
5.61	1.22
9.40	1.93
15.09	2.61
20.38	3.17
24.85	3.56
30.13	4.04

VKHB 11-06-a

October 18, 2000

LED #9 Large Green (NSPG500S) (w/resistor) (w/o reading)

Current (mA)	Power (mW)
4.34	0.97
9.67	1.73
14.90	2.39
19.71	3.13
24.80	3.45
30.14	4.37

LED # 11 Large Green (NSPG 500S) (w/ resistor) (w/o reading)

Current (mA)	Power (mW)
5.10	1.07
10.80	2.07
15.60	2.62
19.60	3.12
25.56	3.65
30.28	4.15

LED #12 Large Green (NSPG 500S) (w/ resistor) (w/o reading)

Current (mA)	Power (mW)
5.90	1.35
11.38	2.24
14.58	2.53
19.34	3.34
24.60	3.87
30.60	4.23

✓KHB
11-06-00

LED #13 Large Green (NSPG500S) (w/ resistor) (w/o casing)

Current (mA)	Power (mW)
4.42	1.06
9.40	1.91
15.28	2.57
20.85	3.17
25.36	3.70
30.25 ~~29.65~~	~~3.52~~ 4.23

LED #14 Large Green (NSPG500S) (w/ resistor) (w/o casing)

Current (mA)	Power (mW)
5.09	1.15
16.94	2.16
14.86	2.75
19.85	3.22
24.50	3.89
29.82	4.18

LED #15 ~~Large Green (NSPG500S) (w/ resistor) (w/o casing)~~ Large Bluish-Green (NSPE590S)

Current (mA)	Power (mW)
4.99	0.15
9.74	0.24
14.58	0.37
19.79	0.46
24.60	0.54
30.30 ~~24.38~~	~~0.55~~ 0.61

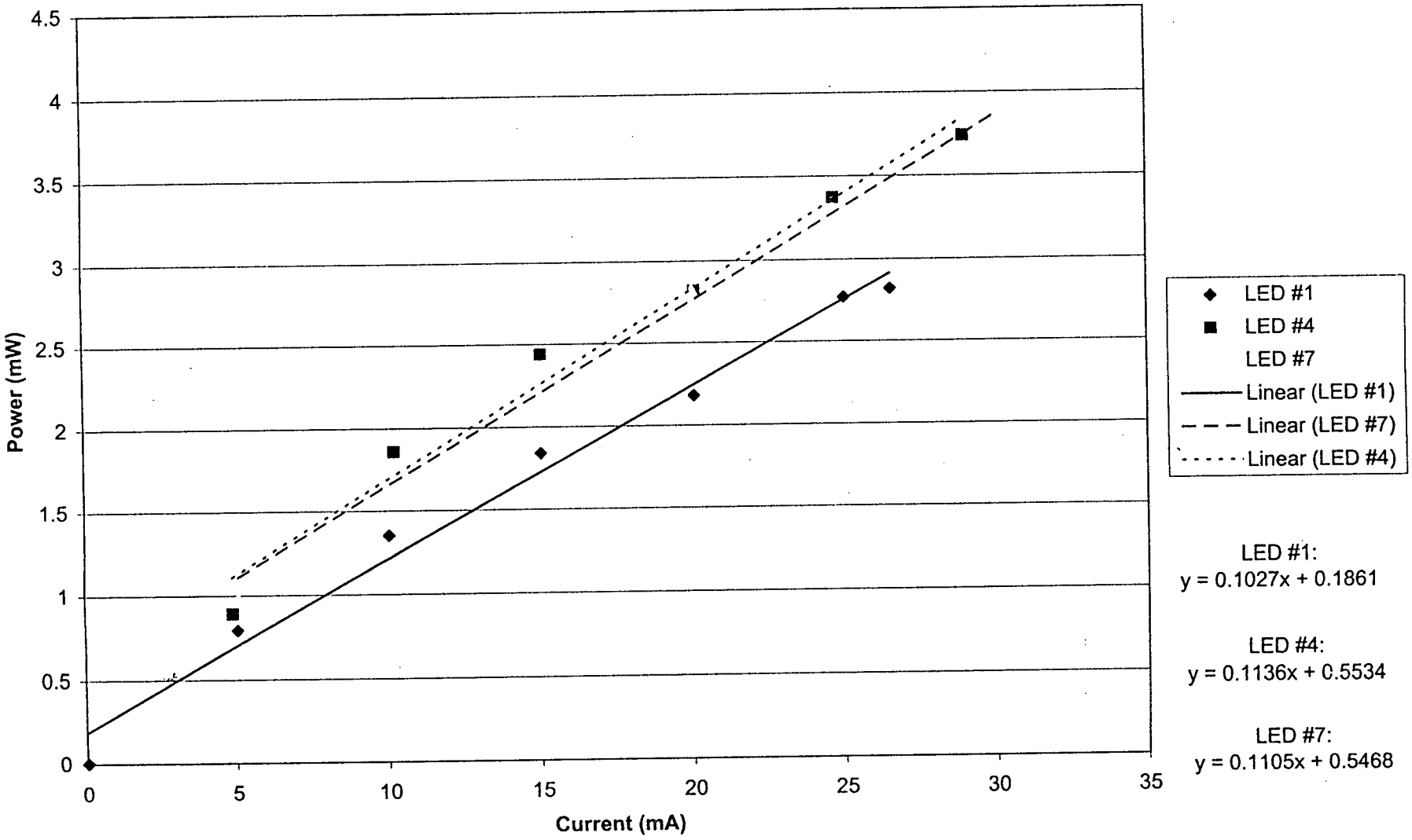
YKB
11-06-00

* At 30mA, we see a drop (steady) in Power.

12/18/00

KHB 11-08-00

LED Power Output



LED #1:
y = 0.1027x + 0.1861

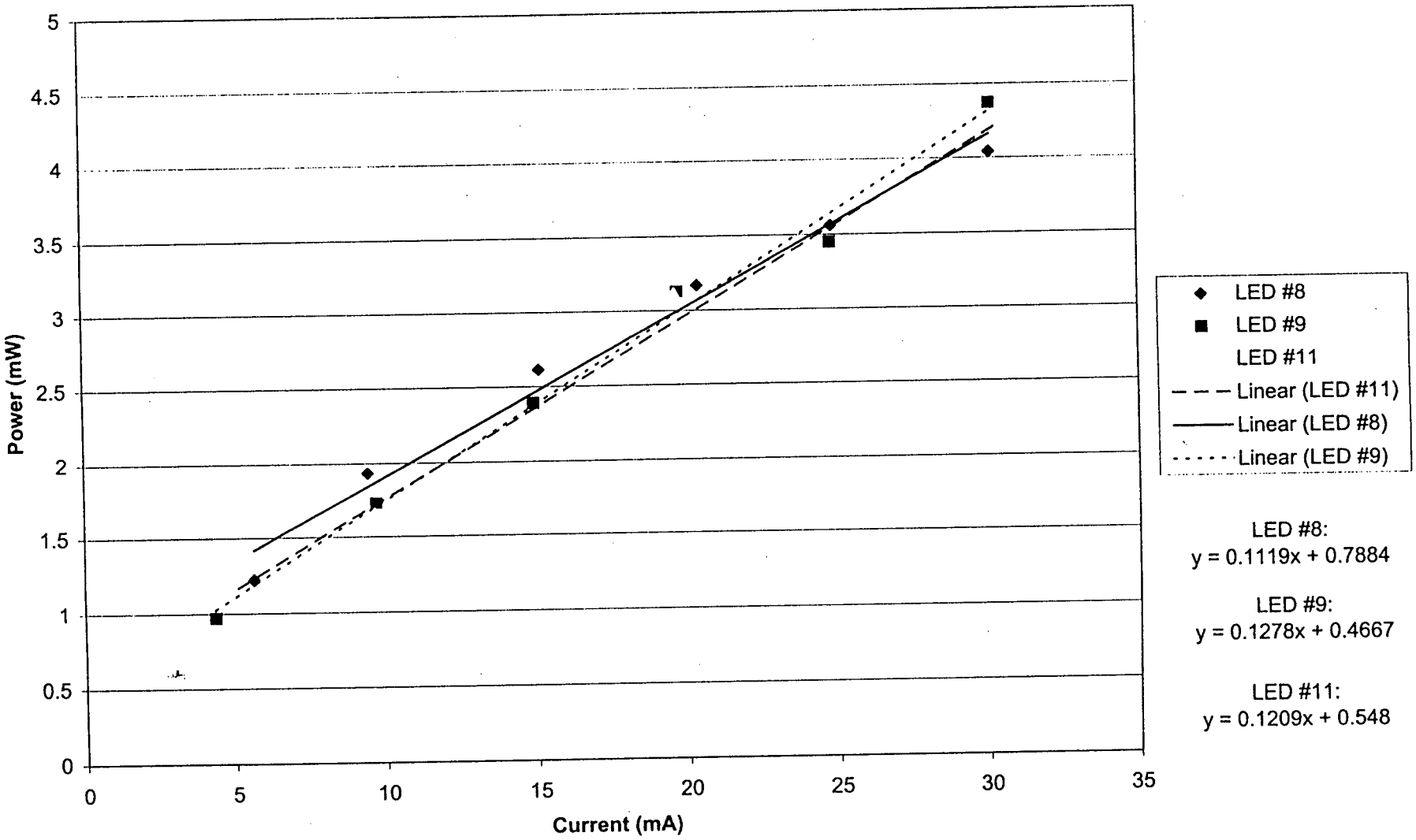
LED #4:
y = 0.1136x + 0.5534

LED #7:
y = 0.1105x + 0.5468

9/18/00

JKB
11-06-00

LED Power Outputs



LED #8:
y = 0.1119x + 0.7884

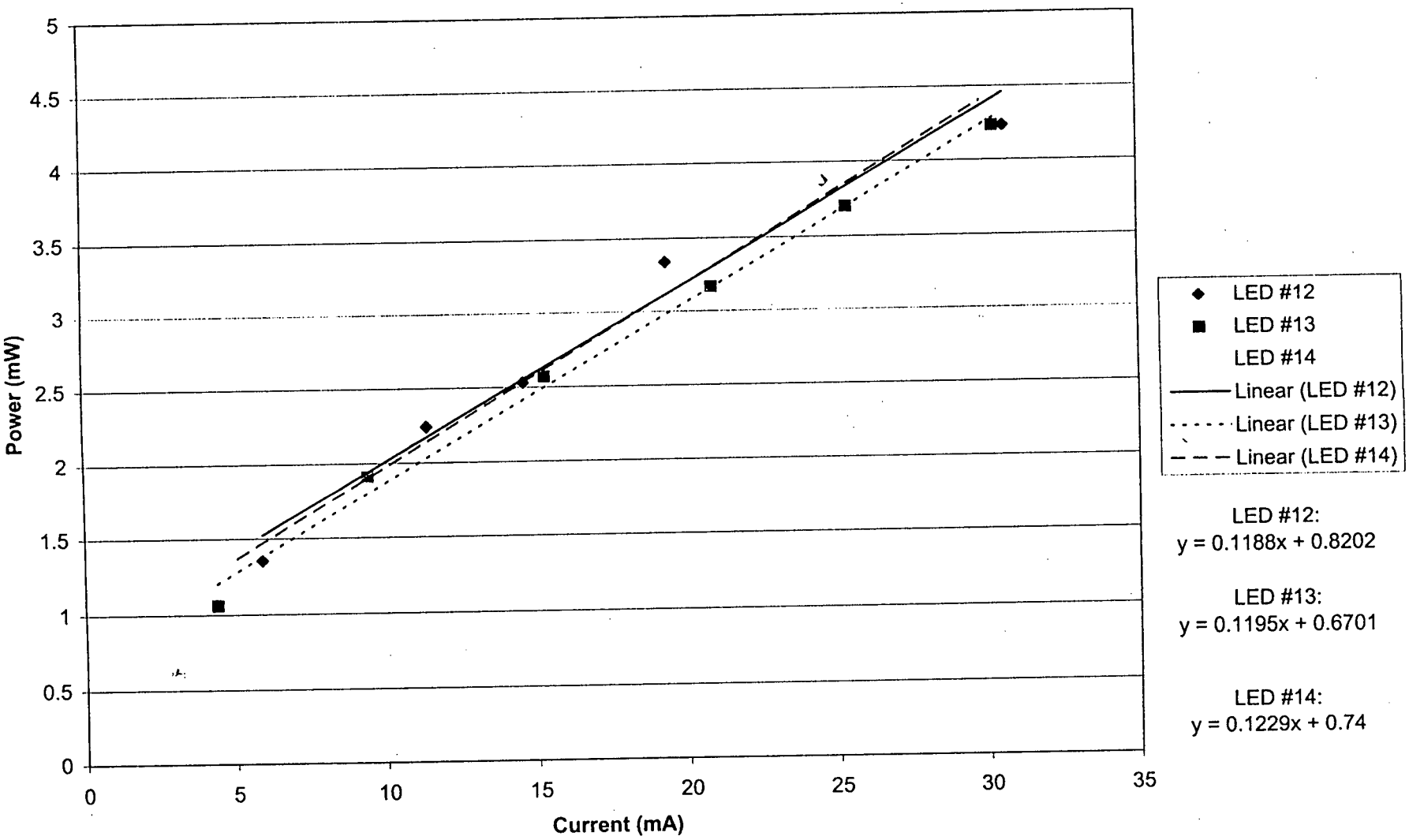
LED #9:
y = 0.1278x + 0.4667

LED #11:
y = 0.1209x + 0.548

10/16/00

JK4B 11-06-00

LED Power Output



LED #12:
y = 0.1188x + 0.8202

LED #13:
y = 0.1195x + 0.6701

LED #14:
y = 0.1229x + 0.74

October 19, 2000

Large Green LEDs

	Ranking according to fitted slope
LED #1	9
LED #4	6
LED #7	8
LED #8	7
LED #9	1
LED #11	3
LED #12	5
LED #13	4
LED #14	2

Large Bluish-Green LED's

LED #2
LED #5

JKB
11-06-00

October 19 2000

To Do: Fit one (not best) LED into brass casing w/ epoxy
(grind off optics & sand down)
Take 3 or so runs @ range of currents to compare.
See if Power drops off @ 30 mA.
→ Test Blue & small Green LED's
→ Make smooth plots of graphs

October 24/25, 2000

USE LED #1 → Remove optics

- Cut off and sanded optics w/ Dremmel Tool
- Epoxy LED#1 into optics casing

Measure Power output from casing

RUN #1 [LED#1] in casing

Current (mA)	Power (mW)
5.08	0.43
10.37	0.77
15.71	1.07
19.67	1.22
25.42	1.43
28.53	1.62

Did see a decrease in power @ 30mA

RUN #2

Current (mA)	Power (mW)
4.59	0.40
11.01	0.82
16.47	1.11
20.00	1.26
24.38	1.46
29.50	1.62

Did see ↓ in P @ 30 mA

VK+B 11-06-00

RUN #3

Current (mA)	Power (mW)
6.25	0.51
9.89	0.74
14.45	0.93
19.57	1.25
25.06	1.49
29.90	1.66

Did see drop in P @ 30 mA

VK+B
11-06-00

Measure inner diameter of casing:

inner casing OD ≈ 0.355" = 9.017

outer casing ID = 0.395 = 10.033 mm

USE LED #12 → Remove Optics

- Cut off & sand optics w/ Dremel Tool
- Epoxy #12 into casing

Measure Power Output

Run #1 [LED #12] in casing

Current (mA)	Power (mW)
4.66	0.43
10.58	0.82
14.83	1.04
20.46	1.28

saw decrease in Power @ 20mA

Run #2

Current (mA)	Power (mW)
5.96	0.51
10.06	0.78
15.66	1.08
20.24	1.28

Run #3

Current (mA)	Power (mW)
4.82	0.44
11.35	0.85
15.53	1.07
20.55	1.30

Julie Wilcox

JKB 12-4-00

November 6, 2008

TEST Flat-pack LED's (w/resistor) (w/o coding)

LED's #15-24

LED #15

Current (mA)	Power (mW)
5.16	0.36
10.09	0.59
15.37	0.83
20.00	1.02

LED #19

Current (mA)	Power (mW)
5.57	0.33
9.80	0.49
14.65	0.65
20.30	0.93

LED #16

Current (mA)	Power (mW)
5.34	0.34
10.06	0.55
15.26	0.76
19.47	0.93

LED #20

Current (mA)	Power (mW)
5.07	0.37
10.95	0.66
14.66	0.93
20.40	1.06

LED #17

Current (mA)	Power (mW)
4.58	0.38
9.95	0.71
15.57	0.98
20.25	1.21

LED #21

Current (mA)	Power (mW)
5.96	0.41
10.42	0.64
15.80	0.91
20.40	1.04

LED #18

Current (mA)	Power (mW)
5.21	0.41
10.40	0.71
15.30	1.03
20.00	1.13

LED #22

Current (mA)	Power (mW)
4.88	0.34
10.70	0.64
14.70	0.93
20.88	1.00

VKB 12-4-08

LED #23

Current (mA)	Power (mW)
5.22	0.43
10.72	0.73
15.26	0.98
20.15	1.12

LED #24

Current (mA)	Power (mW)
4.90	0.39
10.00	0.60
15.28	0.87
19.90	1.04

11/20/00

Blue LED's (Large NSPE590S)

(LED's #25 - #27)

LED #25

Current (mA)	Power (mW)
4.63	0.32
10.66	0.65
15.43	0.92
19.50	1.07

decrease in luminosity @ 20 mA

LED #26

Current (mA)	Power (mW)
4.93	0.30
9.93	0.54
15.24	0.82
19.80	1.07

decrease in Power/luminosity @ 20 mA

LED #27

Current (mA)	Power (mW)
4.58	0.31
9.90	0.59
16.98	0.89
19.96	1.07

decrease in Power @ 20 mA after ~5 sec.

VKB 12-4-00

Jules Kolbo 11/20/00

11/29/00

Small Green LEDs (NSPG300A)

(LED #28 - #34)

LED #28

Current (mA)	Power (mW)
4.76	0.38
10.28	0.61
15.93	0.81
20.10	0.87

decrease in Power @ 20mA after a few seconds

11/30/00 LED = 0.87 mW (NSPE590S)

LED #29 (LED's #25 - #27)

Current (mA)	Power (mW)
4.37	0.30
10.35	0.62
14.97	0.81
20.03	0.99

decrease in Power @ 20mA after a few seconds

LED #30

Current (mA)	Power (mW)
5.29	0.45
10.81	0.67
14.96	0.82
20.24	0.93

decrease in Power @ 20mA after a few seconds

LED #31

Current (mA)	Power (mW)
4.62	0.34
9.64	0.58
15.03	0.77
20.03	0.94

decrease in Power @ 20mA after a few seconds

VKB
12-4-00

LED #32

Current (mA)	Power (mW)
5.10	0.36
10.51	0.58
15.88	0.81
20.33	0.92

decrease in Power after a few seconds @ 20 mA

LED #33

Current (mA)	Power (mW)
5.28	0.40
11.65	0.76
15.42	0.95
20.51	1.09

decrease in Power @ 20 mA after a few seconds

LED #34

Current (mA)	Power (mW)
5.04	0.45
10.46	0.75
15.46	0.99
20.57	1.10

decrease in Power @ 20 mA after a few seconds.

VKB
12-4-00

November 29, 2000

New Large Green LEDs (NSPG500S)

(LED's #36 - #45)

Tested only every ≈ 2mA to 10mA to prevent fatigue.

LED #36

Current (mA)	Power (mW)
1.96	0.52
3.77	0.85
5.93	1.26
7.83	1.37
10.01	1.70

LED #37

Current (mA)	Power (mW)
1.87	0.43
3.89	0.95
6.24	1.25
8.07	1.53
10.16	1.74

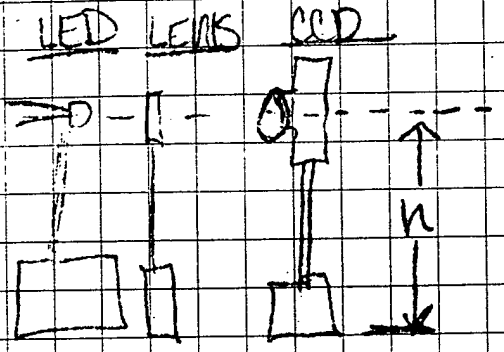
LED #38

Current (mA)	Power (mW)
1.91	0.48
3.96	1.00
5.99	1.34
7.98	1.61
10.10	1.83

KB
12-4-00

January 18, 2001

Setup for testing cylindrical lenses:



Take pics of illumination
Change
- dist. of LED fr. Lens
- dist of camera fr. LENS
- placing of LENS

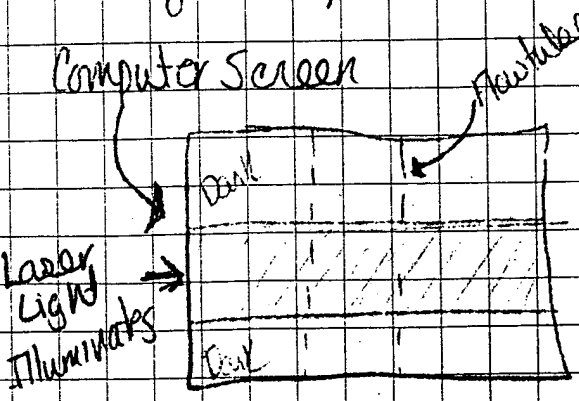
January 23, 2001

Align instruments → LED, Optics, CCD
- Waiting for Power Source

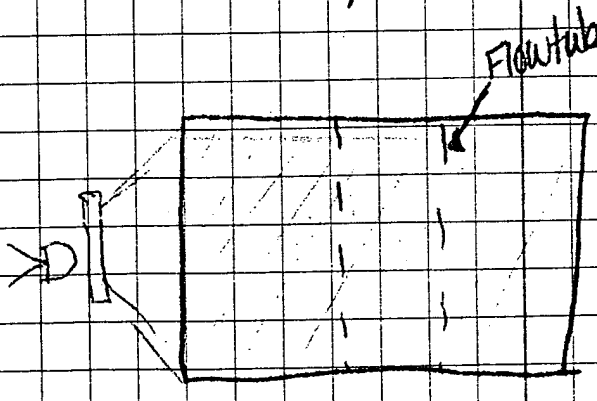
h → Up 21 1/2 cm aligned

January 25, 2001

Before (w/ Laser):



Need w/ LED:



VKB
2-02-01

* Illuminate entire flowtube

January 30, 2001

Pictures w/ CCD camera proved futile w/ this setup → Either No Light or All Light.

Shined LED against black background to see shape of beam (All done w/ Lg Green LED #12 w/o sanded optics in brass casing.)

(1) FLAT back View

Orientation

Left Side View

Beam LED Optics

Beam Shape:

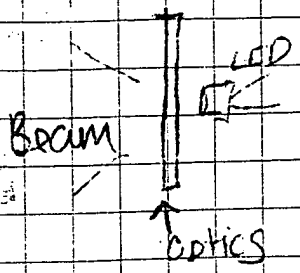
LIGHT

(2) Orientation:

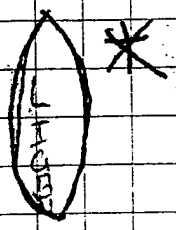
Back View

Side View

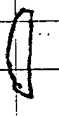
JKB
2-02-01

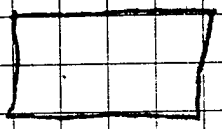


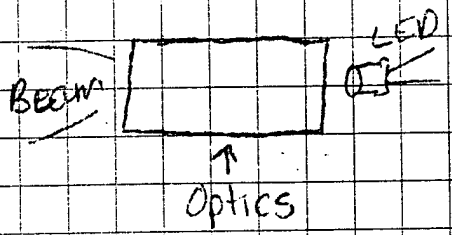
BEAM SHAPE:



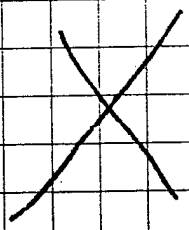
(3) Orientation


Back View


Side View

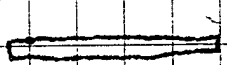


BEAM SHAPE:

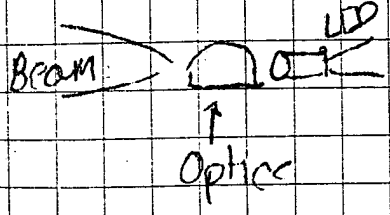


NOT GOOD

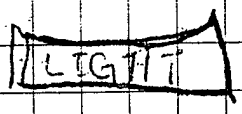
(4) Orientation


Back View


Side View



BEAM SHAPE:

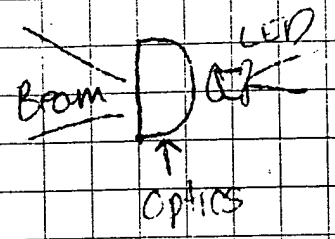


With lots of extra Background Light.

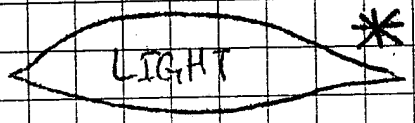
VKB
2-02-01

(5) Orientation

* rounded Back View Side View



BEAM SHAPE:



* Can increase height & width by position of LED.

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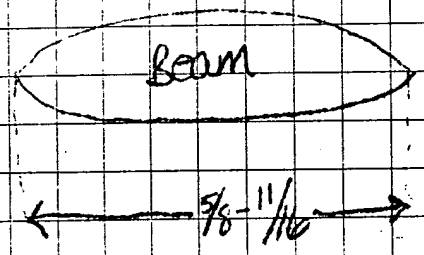
2-02-01

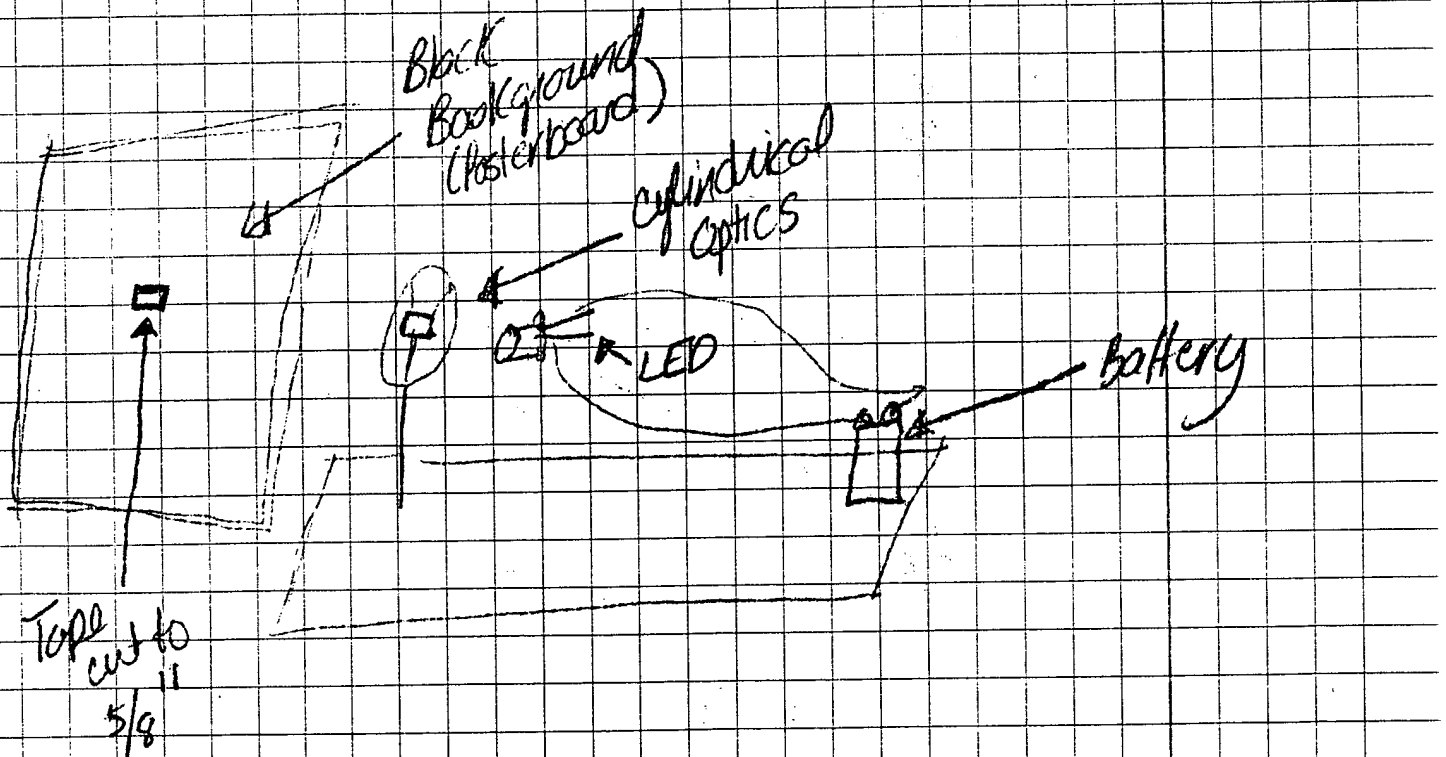
February 6, 2001

Size (length of) flow-tube illumination

[5/8" - 11/16"]



Experiment to find beam LED distance from flow tube



- Put LED butted up against optics.
- Shine LED beam w/ optics on tape
- Move posterboard forward/backward until beam is just the size of the tape.

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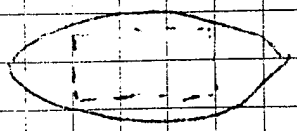
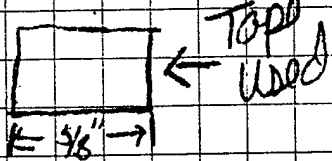
3-01-01

Configuration #1

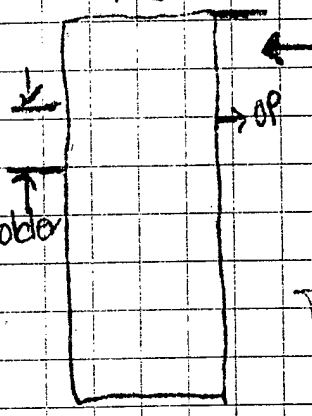
* With this experiment, posterboard also butted up against optics holder provide ample light.





* Make outline on posterboard of beam shape & take measurements

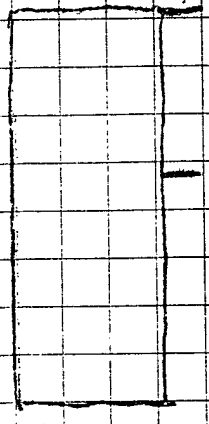
① PB



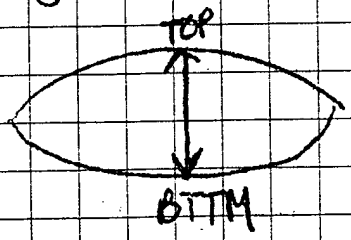
PB = Posterboard Outline

DEPTH MEASURED (PBOP) = 9/16"

② TOP



(highest portion → middle)



HEIGHT MEASURED (TOP BTTM) = 14/16" = 7/8"

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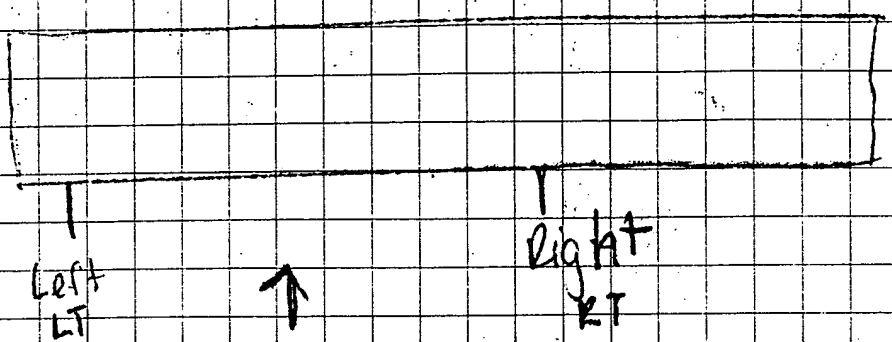
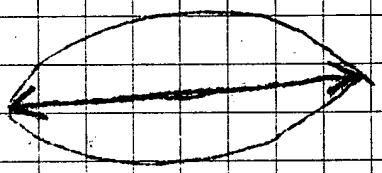
3-01-01

(3)

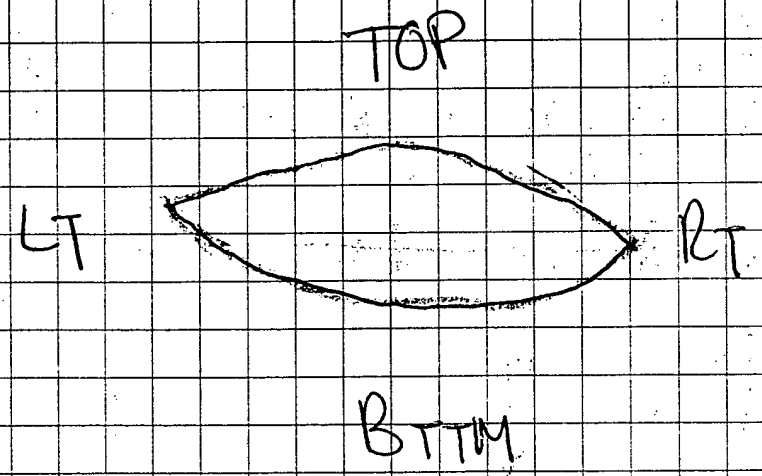


Length of beam @ depth of ~~9/16~~ 9/16"



LENGTH MEASURED = 2 1/2"
(LTRT)

(4) Traced Version of beam outline:



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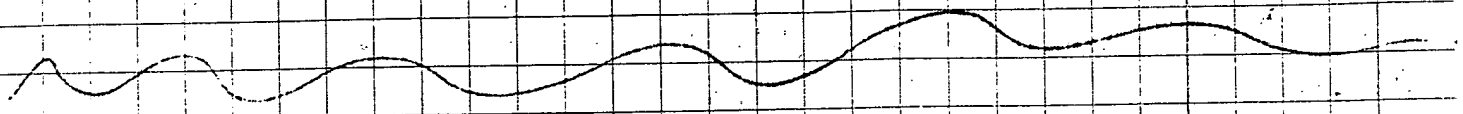
3-01-01

February 15, 2001

* Need steady rectangular beam of



9 Volt battery → $V = iR$

$i = V/R = 9V/1000\Omega = 9mA$

12 V battery → $i = 12V/1000\Omega = 12mA$

Should only use up to 20 V power source!

JKB
3-01-01

EXHIBIT N

Date: Wed, 21 Feb 2001 10:50:14 -0700
From: "David F. Langiulli" <DLang@uwyo.edu>
Subject: US Patent Filing for UW 00-004 Entitled LED Flow Cytometer Illumi
nation (Johnson)
To: "Jennifer Bales (E-mail)" <bales@mbj-law.com>
Cc: "Paul E. Johnson" <PJohnson@uwyo.edu>,
"William A. Gern" <WillGer@uwyo.edu>,
"Rodney P. Lang" <RLang@uwyo.edu>, Roger Wilmot <RWilmot@uwyo.edu>
Importance: high
X-Priority: 1

Jennifer:

Good speaking with you this morning. Please see Letter of Engagement below. As we discussed, you will review the initial application filed by Winslow to see if there was any mention of LED's in order that we might be able to take advantage of that priority date.

Please call me if you have any questions.

DL

David Langiulli, Director
University of Wyoming Research Products Center
P.O. Box 3672, Laramie, WY 82071
Tel: 307-766-2509 Fax: 307-766-2530 Cell: 307-760-1962
Email: Dlang@uwyo.edu
Web Site: http://www.uwyo.edu/rpc
Helping Wyoming Inventors/Entrepreneurs Identify, Protect & Commercialize Intellectual Property

Letter of Engagement for the Patent Prosecution of UW/Softray Invention Entitled:

"LED Flow Cytometer Illumination" (Paul Johnson)
UW Technology ID No. : 00-004

Dear Jennifer:

This is to confirm that the University Of Wyoming (UW), with authority from the Office of University Counsel, has retained you to file a United States Patent Application on the invention of Professor Paul Johnson entitled "LED Flow Cytometer Illumination". This application should be filed no later than March 2nd, 2001. Please let us know immediately if you will not be able to meet this deadline.

As per out discussion, it is our expectation that total billings for all costs associated with preparing and filing the application, drawings, oath of declaration, and an assignment (jointly between UW and Softray), including attorney time, fees, and any out of pocket expenses, will not exceed $4,500. Please inform us immediately if you anticipate expenses to exceed this amount. All invoices should be itemized, and should be forwarded to me at the Wyoming Research Products Center (RPC). In order to properly access the scope of the work accomplished and keep our files current, it is essential that all related documents be in UW's possession. Any documents

not previously forwarded must be attached to the invoice. Attorney invoices will not be considered for payment until our office is in receipt of the necessary papers. Following approval of the expenditures, RPC will forward the invoice to the appropriate internal or external party(s) for payment.

Please note that in the absence of a license or obligation to license inventions to large entity(s), UW is eligible to claim small entity status and thereby pay reduced patent office fees. This is the case for this invention. Please be sure that UW pays fees at the reduced rate.

As you are aware, RPC has overall responsibility for the management and licensing of the UW patent portfolio. We therefore ask that you provide this office with (1) a copy of the application and filing receipt, (2) an original assignment of the invention jointly to UW and Softray at the time of filing the patent application, (3) copies of all correspondence with the inventors, (4) copies of all correspondence with the Patent Office, including office actions, responses and amendments, and (5) copies of other documents that may reasonably be of value. It is our intention that the UW file be as complete as that which you maintain in your own office. It is only with such complete information that we can professionally advise our faculty inventors and knowledgeably discuss the invention with potential licensees. Please also copy the inventors on any correspondence relative to them i.e., office actions, responses and amendments.

UW has assigned Technology ID Number 00-004 to this technology. Please be certain to include this number prominently on all correspondence and copies of documents forwarded to RPC regarding this case. Failure to include this ID number on relevant documents, particularly invoices, may substantially delay payment.

Although this office is responsible for overseeing the patenting process, it is not our intention to intercede in or interfere with the open communication between UW inventors and their patent counsel. Please feel free to communicate directly with the inventors regarding all technical matters of the application as you feel necessary for the proper and expedient prosecution of the case. Of course, if you should experience any difficulty in obtaining necessary information, documentation, or other assistance from the inventors, please let us know so that we can actively seek to alleviate such difficulties. Furthermore, all formal matters and decisions regarding conversion to PCT, continuation or abandonment, division, appeal, and costs should be brought to the attention of RPC rather than to the inventors.

You were selected by UW because of your unique technical background and level of skill. Therefore, you will personally attend to the drafting and prosecution of all technical aspects of the patent application(s) contemplated, with staff support on formal and administrative aspects. Any change in this commitment must be approved by UW.

Upon receipt of an official office action, please forward it to RPC, with a copy to the inventors for their comments. If you do not receive notice to the contrary from RPC within 30 days, you may assume that UW wishes you to respond to the office action. It is expected that your billings for preparing and filing a response will be in addition to that billed for initial filing, and that billing for your services will be at the same hourly rate.

We look forward to working with you on this project.

Sincerely,

David Langiulli, Director
Wyoming Research Products Center

EXHIBIT M

Status: U
Date: Fri, 01 Nov 2002 15:12:13 -0700
From: "Paul E. Johnson" <PJohnson@uwyo.edu>
Subject: FW: Patents
To: bales@mbj-law.com
Thread-Topic: Patents
Thread-Index: Ab/8DrlddLH4PWezEdSu6ABAM9pw7gAsUwFAAAJ5+4CFCILVoAAAGhYg
X-OriginalArrivalTime: 01 Nov 2002 22:12:13.0153 (UTC)
FILETIME=[BAD7B510:01C281F3]

-----Original Message-----
From: Paul E. Johnson
Sent: Friday, November 01, 2002 3:09 PM
To: Paul E. Johnson
Subject: FW: Patents

-----Original Message-----
From: David F. Langiulli
Sent: Wednesday, August 02, 2000 3:32 PM
To: Paul E. Johnson
Subject: RE: Patents

No problem. As I have said, I know of a couple of good attorneys that we can use.

DL

David Langiulli, Director
University of Wyoming Research Products Center
P.O. Box 3672, Laramie, WY 82071
Tel: 307-766-2509 Fax: 307-766-2530 Cell: 307-760-1962
Email: Dlang@uwyo.edu
Web Site: http://www.uwyo.edu/rpc

> -----Original Message-----
> **From:** Paul E. Johnson
> **Sent:** Wednesday, August 02, 2000 2:28 PM
> **To:** David F. Langiulli
> **Subject:** RE: Patents
>
> David,
>
> Thanks for being patient. I talked to Winslow about the
> possibility of dropping the LED patent. He will tell me in
> two weeks whether or not he has too much on his plate. Right
> now I am inclined to have another party work on that patent.
> Let's try to make a plan of action shortly, by the end of
> August in any case.
>
> Paul
>

> -----Original Message-----

> **From:** David F. Langiulli
> **Sent:** Tuesday, August 01, 2000 5:12 PM
> **To:** Paul E. Johnson
> **Subject:** Patents
>
> In addition to talking about our strategy and tactics for the
> Dakota meeting, we should talk about the status of the
> patents with Winslow. I recently got a good referral of a EE
> patent attorney.
>
> DL
>
> _____
> **David Langiulli, Director**
> **University of Wyoming Research Products Center**
> **P.O. Box 3672, Laramie, WY 82071**
> **Tel: 307-766-2509 Fax: 307-766-2530 Cell: 307-760-1962**
> **Email: Dlang@uwyo.edu**
> **Web Site: http://www.uwyo.edu/rpc**
>
>